

Poster Session 1

Basic immunology

413

New express immunochromatographic assay in epidermal growth factor concentrations determinationYastrabava, H¹; Karpenka, T¹; Tsishkevich, M¹; Piven, N¹; Lukhverchyk, L¹; Burakovski, A¹¹Laboratory of Medical Microanalysis, Institute of Bioorganic Chemistry of Belarus National Academy of Sciences, Minsk, Belarus

Background: Epidermal growth factor (EGF) is one of the perspective clinical, diagnostic, pathogenetic and prognostic markers in various forms of cancer, associated with its overexpression. Abnormalities in signaling system of EGF and its receptor cause more than 70% of all malignant tumors. The EGF concentration estimation (its overexpression) can be performed only with the use of modern highly sensitive methods, which includes immunochemical assays, and in particular, the immunochromatographic assay.

Method: A new express immunochromatographic assay of epidermal growth factor in serum was elaborated.

Results: The developed method based on the usage of two types of specific antibodies and multimembrane complex – test strips, on which monoclonal antibodies to EGF (analytical zone) and anti-specific antibodies controlling the validity of the test (control zone) were immobilised. The method has the following analytical characteristics: sensitivity (the detectable minimum of EGF concentration) – 1 ng/ml, analysis duration – 10–15 min. The approbation of the developed method was carried out on the clinical material from the patients with cancer of various localisation with obtained ELISA data comparison. A comparative analysis of two methods has shown the comparability of the obtained data that indicate the clinical and diagnostic value of new method and confirm the possibility of its usage in medical practice.

Conclusion: The new method allows with high accuracy and specificity to detect an increased EGF concentration and to diagnose initial stages of cancer development, associated with its overexpression. It also provide researchers with express quantitative criterion of the pathological process manifestation control, therapy effectiveness and prognosis of the disease.

415

The cytotoxicity of aflatoxin B₁ in human lymphocytesAl-Hammadi, S¹; Marzouqi, F¹; Al-Mansouri, A¹; Shahin, A²; Al-Shamsi, M²; Mensah-Brown, E³; Souid, A-K¹¹Department of Pediatrics, UAE University, Al-Ain, United Arab Emirates; ²Medical Microbiology, UAE University, Al-Ain, United Arab Emirates; ³Anatomy, UAE University, Al-Ain, United Arab Emirates

Background and Method: Aflatoxins are naturally occurring carcinogenic and immunosuppressive compounds. In this study, phosphorescence oxygen analyzer that measured glucose-driven cellular respiration (mitochondrial oxygen consumption) as a function of time was used to monitor the effects of aflatoxin B₁ on the human lymphocyte. Oxygen concentration [O₂] was determined from the phosphorescence decay rate of Pd(II)-meso-tetra-(4-sulfonatophenyl)-tetra-benzoporphyrin.

Findings: Cyanide inhibited cellular O₂ consumption, confirming the oxidation occurred in the respiratory chain. In sealed vials, O₂ consumption was linear with time, verifying its zero-order kinetics. The rate of respiration (μM O₂/min) was, thus, the negative of the slope of [O₂] vs time. In the presence of aflatoxin B₁, O₂ consumption by peripheral blood mononuclear cells was unaffected in the first hour; thereafter, it became exponential with time. This effect was blocked by the pan-caspase inhibitor benzyloxycarbonyl-val-ala-DL-aspartate-fluoro-methylketone (zVAD-fmk), confirming caspases were responsible for the mitochondrial disturbances. Intracellular cleavage of the caspase-3 substrate N-acetyl-aspartate-glu-val-aspartate-7-amino-4-methyl coumarin (Ac-DEVD-AMC) was noted within 2 h of incubation with aflatoxin B₁; zVAD-fmk blocked the release of AMC moiety, confirming caspases were responsible for the cleavage. Apoptosis was also monitored by flow cytometry, using phosphatidylserine exposure and membrane permeability to propidium iodide (PI). The number of annexin-V positive cells increased as a function of aflatoxin B₁ concentration and incubation time. At the highest concentration employed, a significant number of the mononuclear cells become necrotic (annexin-V⁺PI⁺) after 24 h.

Conclusion: The results clearly show that aflatoxin B₁ is toxic to human lymphocytes and its effect is mediated, at least in part, by caspase activity.

416

C-reactive protein and antiendotoxin immunity in patients with chronic kidney disease receiving hemodialysisBiloglazov, V¹; Klimchuk, A¹; Gordienko, A¹; DuBuske, L²¹Crimean State Medical University, Simferopol, Ukraine; ²Immunology Research Institute of New England, Gardner, MA, United States

Background: Uncontrolled inflammation may be associated with cardiovascular disease in patients receiving hemodialysis. This study investigated the level of C-reactive protein and anti-endotoxin immunity in patients with chronic kidney disease who are treated with hemodialysis (HD).

Method: This study was done in two stages over a 4 year interval. Initially 46 patients 30–68 years old with chronic kidney disease receiving HD were assessed. C-reactive protein and levels of serum anti-endotoxin antibodies (anti-ET IgA, anti-ET IgM, anti-ET IgG) were determined by ELISA. Blood samples were obtained from patients before HD. In 4 years in the second stage, only 32 patients were studied again, as 14 (30.4%) had died. Seven patients (15.2%) died during the first year after start of the study. Levels of anti-ET IgA, anti-ET IgM, anti-ET IgG from 38 healthy individuals served as controls.

Results: The seven patients who died in the first year after the start of the study had levels of C-reactive protein 3.1 fold ($P < 0.001$) greater than healthy controls and in 1.2 fold ($P = 0.019$) greater than other subjects. Anti-ET IgA, anti-ET IgM, anti-ET IgG were similar to healthy controls and anti-ET IgG was 1.7 fold ($P = 0.031$) and anti-ET IgM 2.1 fold ($P = 0.045$) less than other study subjects. Chronic kidney disease patients receiving hemodialysis had C-reactive protein levels 2.5 fold ($P < 0.001$) greater than healthy controls with a 4.6 fold ($P < 0.001$) increase over 4 years. Anti-ET IgG increased by 2.6 fold ($P < 0.001$) more than in healthy controls. During 4 years,

levels of anti-ET IgA, anti-ET IgM, anti-ET IgG in the HD patients did not change.

Conclusion: Increased C-reactive protein and decreased anti-ET IgM, anti-ET IgG predicted a lethal outcome in 7 HD patients. Four years of monitoring chronic kidney disease hemodialysis patients showed a 4.6 fold increase in C-reactive protein ($P < 0.001$) but no change in indicators of humoral anti-endotoxin immunity.

418

Content and immunophenotype of antigen presenting cells in patients with multiple drug resistant pulmonary tuberculosis

Titov, LP¹; Ramanava, IU¹; Hancharou, AY¹; Solodovnikova, VV²; Vetushko, DA²; DuBuske, LM³

¹Republican Scientific and Practical Center for Epidemiology and Microbiology, Minsk, Belarus;

²Republican Scientific and Practical Center for Pulmonology and Physiology, Minsk, Belarus;

³Immunology Research Institute of New England, Gardner, MA, United States

Background: Functional impairment and improper maturation of the antigen-presenting cells (APC) may lead to severe pulmonary tuberculosis (TB). This study investigates the content and immunophenotype of APC from the patients with severe multiple drug resistant pulmonary tuberculosis (MDR TB).

Method: Peripheral blood samples were obtained from patients with MDR TB ($n = 15$) matched by age. The control group (C) included 15 healthy subjects. Analysis of content and immunophenotype of blood monocytes, and monocytic and plasmacytoid dendritic cells (mDC, pDC) was performed by flow cytometer using four-color antibody panels.

Results: Increase in absolute number and relative content of monocytes in the peripheral blood of patients with MDR TB compared with the C ($P = 0.001$ and $P = 0.02$ respectively) was observed. The monocyte population was characterised by significantly decreased expression of CD80 in the MDR TB patients group compared to the C ($P = 0.000041$). The absolute number of pDC in patients with MDR TB was significantly decreased vs the C ($P = 0.0001$) while mDC number was unchanged ($P = 0.087$). Numbers of mDC were greater than pDC in the MDR TB patients, while in the C numbers of pDC were more than mDC. However relative numbers of both mDC and pDC were decreased in the patients with MDR TB compared to the C ($P = 0.005$ and $P = 0.0006$ respectively). The decrease of CD80 expression by mDC (MDR TB –

2.17(0.71–4.73)%; C – 14.2(2.7–37.4)%; $P = 0.0004$) and reduction of the intensity expression of co-stimulatory molecules CD86 by mDC and pDC ($P = 0.03$ and $P = 0.0005$ respectively) were noted in MDR TB patients. There were no changes in the expression of CD54 molecules by either pDC and mDC in MDR TB patients.

Conclusion: The increased number of monocytes and mDC in the peripheral blood in association with decreased expression of co-stimulatory molecules on their surface probably reflects damage not only in functional ability, but also in migration DC from the blood into the regional lymph nodes and lungs of MDR TB patients.

419

Serum adenosine deaminase activity and T cell IFN-gamma production in patients with tuberculosis

Yanovich, OO¹; Titov, LP¹; Shpakovskaya, NS²; Dyusmikeeva, MI²; DuBuske, LM³

¹Republican Scientific and Practical Center for Epidemiology and Microbiology, Minsk, Belarus;

²Republican Scientific and Practical Center for Pulmonology and Physiology, Minsk, Belarus;

³Immunology Research Institute of New England, Gardner, MA, United States

Background: Adenosine deaminase (ADA) is important enzyme for immunoinflammatory responses and serves as a marker of activated leukocytes. ADA is required for proliferation and differentiation of T-lymphocytes. Its deficiency mainly affects T cell activation and cell-mediated immunity. This investigation assesses serum ADA activity and IFN-gamma production *in vitro* by T cells in patients with tuberculosis.

Method: Serum adenosine deaminase levels were measured with a colorimetric method described by Giusti in patients with tuberculosis ($n = 22$) and healthy controls ($n = 20$). For *in vitro* stimulation PBMCs (1×10^6 /ml) were cultured in RPMI-1640 medium with L-glutamine and 10% serum in the presence of 20 μ g/ml PHA using 96-well plates. Culture supernatants were harvested after 1 day of cell culture. IFN-gamma levels were determined by enzyme immunoassay kit (Vector-Best, Russia).

Results: Patients with tuberculosis had significantly higher levels of ADA in serum (25.2 ± 3.7 U/l) than healthy controls (13.2 ± 0.9 U/l), ($P < 0.05$). Patients with tuberculosis were divided into two groups: Group1 – ADA level in the normal range or was moderately increased (up to 23 U/l), the Group 2 – ADA activity significantly above normal (> 23 U/l). *In vitro* IFN-gamma production by lymphocytes of tuberculosis patients in these two groups

was investigated. In Group1, spontaneous levels of IFN-gamma were less compared with Group 2 (8.8 ± 3.2 vs 18.3 ± 4.8 pg/ml). After PHA-stimulation the same tendency was noted 267.7 ± 102.8 pg/ml in the Group 1 and 418.4 ± 196.5 pg/ml in Group 2. In Group 2, 30% of patients had decreased IFN-gamma production after PHA stimulation, that significantly different from Group1 (30% vs 0%, $P < 0.05$).

Conclusion: Elevated levels of ADA activity occur in the serum patients with tuberculosis and indicate an association between ADA activity and T cell function.

420

Characterisation of mucosal immunity are correlated to inflammation in infertile patients with chronic abacterial prostatitis

Drannik, GN¹; Gorpynchenko, II¹; Poroshina, TV¹;

Drannik, AG²; Kurchenko, AI³; DuBuske, LM^{4,5}

¹Institute of Urology, AMS of Ukraine, Kiev, Ukraine;

²McMaster University, Hamilton, ON, Canada; ³National

Medical University, Kiev, Ukraine; ⁴Immunology Research Institute of New England, Gardner, MA,

United States; ⁵The George Washington University School of Medicine, Washington, DC, United States

Background: Cytokines and other immune factors are intrinsically involved in reproductive physiology with local or systemic perturbation of these factors due to inflammation or infection negatively affecting fertility.

Method: Semen samples from 20 healthy males as well as 136 infertile patients with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) were assessed by ELISAs to determine the levels of sIgA, SLPI, PSA, IL-8, TNF- α , IL-17, and TGF- β 1. After liquefaction, semen microscopy was performed to analyze sperm concentration and percentage of motility using WHO classification. Smears of neat semen were also prepared for sperm morphology assessment. Spearman rank correlation analysis was performed to assess interrelations between morphological abnormalities of sperm as well as cytokines and other immunologic factors in the seminal plasma from infertile men with chronic abacterial prostatitis.

Results: CP/CPPS patients had higher concentrations of sIgA, SLPI, IL-8, TNF- α and reduced levels of TGF- β 1 in ejaculate, compared to healthy subjects. A direct correlation between PSA (rK = 0.09), SLPI (rK = 0.116) and percentage of motile sperm in category 'a' ($P < 0.05$) was noted in the healthy group; in CP/CPPS patients such correlation ($P > 0.05$) was absent. CP/CPPS patients had an inverse correlation ($P < 0.05$) between concentrations of TGF- β 1 in the ejaculate and the relative

amount of abnormal sperm morphology (head), ($rK = -0.266$); a direct correlation between IL-17 and the relative number of actively motile sperm with progressive movement ($rK = 0.428$); and an inverse correlation between IL-17 and fixed sperm category 'd' ($rK = -0.366$), $P < 0.05$.

Conclusion: As sIgA, SLPI, PSA, IL-6, IL-8, TNF- α and TGF- β 1 in the ejaculate are mainly produced by epithelial cells of the prostate ducts, immunological changes in local mucosal immunity may promote the formation or advancement of infertility in patients with CP/CPPS.

421

An erythmogenic strategy of narrowband ultraviolet B (311 nm) therapy increased overall immunosuppression in comparison to a sub-erythmogenic strategy as treatment for psoriasis

Babanin, VA¹; Pritulo, OA¹; Bisyuk, YA¹; DuBuske, LM²

¹Crimean State Medical University, Simferopol, Ukraine; ²Immunology Research Institute of New England, Gardner, MA, United States

Background: Psoriasis affects 1–2% of the European population. Narrow-band ultraviolet B (311 nm) therapy causes transient immunosuppression in psoriasis patients.

Method: Ninety-six patients with psoriasis were divided into 2 groups:

- (1) the comparison group of 27 patients using only standard treatment while
- (2) 69 patients used NB-UVB (311 nm) therapy.

The control group included 28 healthy subjects. NB-UVB was administered three times a week using erythmogenic and sub-erythmogenic strategies starting at 70% of the minimal erythema dose. Cytokine levels in serum were determined on days 1, 6 and 12 weeks by ELISA. At day 21, the study group was divided into four groups:

Group 1–20 patients (regression of index PASI over 50%, erythmogenic strategy);

Group 2–21 patients (regression of index PASI over 50%, sub-erythmogenic strategy);

Group 3–14 patients (regression of index PASI <50%, erythmogenic strategy) and

Group 4–14 patients (regression of index PASI <50%, sub-erythmogenic strategy).

Results: On day 1 the levels of TNF- α , IL-6, IL-22 in the study (TNF- α – 26.82 ± 1.86 pg/ml, IL-6 – 15.38 ± 2.35 pg/ml, IL-22 – 36.21 ± 5.09 pg/ml) and comparison group (TNF- α – 25.77 ± 2.14 pg/ml, IL-6 – 12.90 ± 3.22 pg/ml, IL-22 – 37.25 ± 6.20 pg/ml) were significantly greater than the control group (TNF- α – 3.65 ± 0.40 pg/ml, IL-6 – 2.63 ± 0.37 pg/ml, IL-22 – 5.38 ± 0.64 pg/ml, $P < 0.01$). In Groups 1, 2, 3 at 6 weeks, levels of TNF- α

and IL-6 were significantly less ($P < 0.05$) compared to the comparison group (TNF- α – 20.43 ± 1.77 pg/ml, IL-6 – 9.11 ± 4.50 pg/ml), but these cytokines in Group 4 (TNF- α – 19.82 ± 4.14 pg/ml, IL-6 – 7.97 ± 1.77 pg/ml) did not significantly differ ($P > 0.05$). At 12 weeks the cytokine levels did not differ from the control group, except Group 4 where levels (TNF- α – 6.05 ± 1.23 pg/ml, IL-6 – 4.14 ± 1.24 pg/ml, IL-22 – 12.48 ± 1.21 pg/ml) were significantly greater than the controls ($P < 0.05$) and Group 3 (TNF- α – 4.90 ± 0.87 pg/ml, IL-6 – 2.83 ± 0.98 pg/ml, IL-22 – 7.51 ± 1.66 pg/ml).

Conclusion: NB-UVB therapy over 12 weeks significantly reduced the levels of TNF- α , IL-6, IL-22 in comparison to the group of patients receiving only standard treatment. In patients resistant to narrow-band ultraviolet B therapy (regression of index PASI <50%) an erythmogenic strategy of NB-UVB led to increased immunosuppressive effect on systemic inflammation in comparison to sub-erythmogenic phototherapy.

422

Histocompatibility antigens and aggressive phenotypes of patients with oral mucosa lichen planus

Drannik, GN¹; Kurchenko, AI¹; Rehuretska, RA¹; Drannik, AG²; DuBuske, LM^{3,4}

¹National Medical University, Kiev, Ukraine; ²McMaster University, Hamilton, ON, Canada; ³Immunology Research Institute of New England, Gardner, MA, United States; ⁴Allergy and Immunology, The George Washington University School of Medicine, Washington, DC, United States

Background: Lichen planus is the most common and most important systemic immunodependent disease of the oral mucosa. This study investigates the major histocompatibility complex antigens (HLA) related to development of complicated forms of lichen planus.

Method: Seventy-five patients age 18–60 years with lichen planus were assessed. HLA were determined by a standard microlymphocytotoxic test on the Terasaki's planchettes using special panels of anti-HLA serums. The control group consisted of 230 healthy donors.

Results: The expression of HLA – A, HLA – B and HLA – DR antigens by lymphocytes in patients with erosion and ulcer forms of lichen planus by locus A shows a relatively high risk degree of disease in carriers of HLA – A28 expression ($RRI = 2.11$ when $\sigma_1 = 1.33$). This association is absent in patients with the hyperkeratosis form of lichen planus. There is a significant increase of HLA – B8 antigen expression ($RRI = 2.06$ when $\sigma_1 = 1.12$) in

patients with erosion and ulcer forms of lichen planus. Increasing HLA – B27 frequency ($RRI = 2.32$ when $\sigma_1 = 0.6$) in patients with erosion and ulcer forms of lichen planus is of particular importance as HLA – B27 is associated with diseases involving infectious antigens with superantigen properties. There was frequent expression of HLA – DR1 (19.2%), HLA – DR3 (10.2%) and HLA – DR7 (8.2%) antigens.

Conclusion: Steady progress of erosive and ulcerative forms of lichen planus is related to genetic factors including histocompatibility (HLA) Types I and II antigens: HLA – A28, HLA – B8, HLA – B27, and HLA – DR1. The treatment of these aggressive forms of lichen planus needs new approaches.

423

Immune modulatory properties of selected bacterial strains on human dendritic cells

Hiller, J¹; Lehmann, S¹; Back, W²; van Bergenhenegouwen, J^{3,4}; Ring, J⁵; Behrendt, H¹; Schmidt-Weber, C¹; Knippels, L^{3,4}; Garssen, J^{3,4}; Traidl-Hoffmann, C^{1,5}

¹ZAUM- Center of Allergy and Environment, Technische Universität und Helmholtz Zentrum München, Munich, Germany; ²Brewing and Beverage Technology, Technische Universität München, Weihenstephan, Germany; ³Department of Immunology, Danone Research Centre for Specialised Nutrition, Wageningen, The Netherlands; ⁴Division of Pharmacology, Utrecht Institute for Pharmaceutical Science, Faculty of Science, Utrecht University, Utrecht, The Netherlands; ⁵Department of Dermatology and Allergology, Technische Universität München, Munich, Germany

Background: Lactic acid bacteria (LAB) are important immune regulatory microorganisms and are considered to entertain health promoting effects which are of special interest in the view of the growing application of LAB in prevention and treatment of diseases. Aim of the study was to investigate the influence of different bacterial strains on the cytokine release by human monocyte-derived dendritic cells (MoDC).

Method: Immature MoDC generated from peripheral blood monocytes of healthy non-atopic volunteers were stimulated with different *Lactobacillus* and *Bifidobacterium* strains in different concentrations (10^7 – 10^5 cfu/ml). The cytokine profile (including IL-12p70, IL-10, IL-1 β , IL-23, IL-6, TGF- β) of stimulated MoDC was analyzed in supernatants after 24 h.

Results: Stimulation of MoDC with bacteria revealed a highly strain-dependent induction of antiinflammatory IL-10 and pro-inflammatory cytokines such as IL-12p70. High amounts of IL-10 produced by selected bacterial strains points to the induction of potentially functional regulatory T cell responses.

Conclusion: The tested *Lactobacillus* and *Bifidobacterium* strains show specific strain-dependent immune modulatory capacities inducing a more pro- or antiinflammatory cytokine profile in human MoDC. Experiments of this study and future *in vivo* research in this field could contribute to the identification of a potential role for specific lactic acid bacteria as immune modulators in the prevention and treatment of several immune regulatory disorders and open new aspects for the application of bacterial strains as health promoting ingredients in food.

424

State of cytokine status in small airways diseases in children

Mustafayev, IA¹

¹Pulmonology, Research Institute of Lung Diseases, Baku, Azerbaijan

Background: To study the dependence of the state of cytokines on the stage of the pathological process in asthma (BA) and chronic bronchiolitis obliterans (BO).

Method: One hundred and twelve children with BA and 139 patients with BO were observed in age from 1 to 15 years. Studies were conducted in the period of exacerbation and remission. Bronchial obstruction is a common symptom of BA and BO, which differ in etiology and pathogenesis. This was the reason for the diagnostic and therapeutic errors. For each patient was prepared test-card containing the data of family-genetic, obstetric history, prenatal and postnatal periods of life, clinical course, results of radiological, functional and laboratory studies. The degree of air-flow obstruction measured by spirometry. Interleukins IL4, IL8, TNF, γ -interferon were determined by ELISA.

Results: The concentrations of interleukins in BA are at the upper limit, or slightly higher than the norm (IL4- 12.7 ± 0.3 ; IL8-

28, 2 ± 0.6 ; TNF- 5.39 ± 0.12 ; γ -interferon-11, 5 ± 0.3). The marked increase in the level of interleukins observed in BO (IL4-28, 1 ± 0.5 ; IL8-33, 2 ± 0.5 ; TNF-19, 54 ± 0.29 ; γ -interferon 18.7 ± 0.4). Cytokine status in BA was normal in remission (IL- 49 ± 0.2 ; IL-814.9 ± 0.5 ; TNF- 2.92 ± 0.15 ; γ -interferon 7.5 ± 0.8) BO was tendency to their normalisation (IL4- 19.5 ± 0.3 ; IL8- 28.9 ± 0.5 ; TNF- 13.25 ± 0.43 ; γ -interferon- 11.6 ± 0.2).

Conclusion: Cytokine status in BA characterised by a slight increase in levels of interleukins to the upper limit of normal, and its stabilisation in remission. In patients with BO levels of proinflammatory and antiinflammatory interleukins significantly higher than normal. The distinctive feature of the state of cytokines COB is the preservation of their high content in remission, which is manifested in the frequent recurrence and preservation of the inflammatory process in the small bronchi.

427

A good start means everything: *in vitro* Th1 and Th2 cell differentiation is greatly influenced by the initial ratio of naïve and memory CD4+ T cells

Blom, L¹; Poulsen, L.K.¹

¹Copenhagen University Hospital Gentofte, Allergy Clinic, Hellerup, Denmark

Background: Robust *in vitro* systems are important in elucidating mechanisms regarding the heterogenetic nature of *in vivo* allergic immune responses and contribute with knowledge to design good *in vivo* experiments. This study seeks to investigate, by using low tech methods, basic parameters as strength of TCR activation as well as purity of the initial naïve CD4+ T-cells in relation to *in vitro* differentiation of T helper type 1 (Th1) and Th2 cells.

Method: Human CD4+ T-cells and naïve CD4⁺CD45RA⁺CD45RO⁻CD25⁻ T-cells

(>95% or >99%) were isolated, from the PBMC fraction of healthy individuals ($n = 4$), by 1 or 3 negative bead selection rounds. The isolated cells were TCR activated with either 30/30 (weak) or 1000/1000 (strong) ng/ml anti-CD3/CD28 and cultured under Th0, Th1 and Th2 conditions for 1, 3 and 5 days. The cultures were restimulated prior to flow cytometry for intracellular transcription factors, cytokines and supernatant multiplex cytokine analysis.

Results: Using initial cultures of (>99%) naïve CD4+ T-cells but not (>95%) naïve CD4+ T-cells differentiated under Th2 conditions resulted in an expected significantly decreased frequency memory IFN- γ + T-cells at day 1 as well as most GATA3+ T-cells, about 70%, at day 5. Interestingly, comparing the initial (>99%) naïve CD4+ T-cells with the (>95%) naïve CD4+ T-cells cultured under Th2 conditions showed an increased ratio, of more than fourfold, of IL-4/IFN- γ as well as GATA3/Tbet positive cells all days. Furthermore, in the Th2 cultures of initial naïve CD4+ T-cells a tendency towards a higher IL-4/IFN- γ ratio as well as GATA3/Tbet positive CD4+ T-cells was observed comparing weak with strong TCR activation. Th1 cultures of >99% initially naïve CD4+ T-cells showed highest IFN- γ /IL-4 ratio and Tbet/GATA-3 positive cells as well as more Tbet+ T-cells compared with the >95% naïve and CD4+ T-cells. For the Th1 cultures, however, the strength of TCR activation did not influence the ratio IFN- γ /IL-4 and Tbet/GATA-3 positive cells.

Conclusion: Taken together, it is essential to use rigorously purified (>99%) naïve CD4+ T-cells for an optimal *in vitro* differentiation of CD4+ T-cells towards a Th1 or Th2 phenotypes. Additionally, a moderate TCR activation results in better Th2 differentiation.

Poster Session 2

Immune responses in allergy

428

Clusterin inhibits secretion of CCL20 in human bronchial epithelial cells

Kwon, H-S¹; Moon, K-A²; Park, S-Y¹; Yoon, SY¹; Kim, S-J¹; Kim, T-B¹; Cho, YS¹; Moon, H-B¹

¹Department of Allergy and Clinical Immunology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; ²Asan Institute for Life Science, Seoul, Korea

Background: Clusterin, a ubiquitous glycoprotein, has been recently suggested to represent redox state in our bodies as a sensitive indicator of oxidative stress.

Method: Human bronchial epithelial cells (BEAS-2B) were cultured with house dust mite (HDM) extract to evaluate the cellular response to the most common exogenous allergen in airway.

Results: As shown before, production of CCL20, a critical chemokine in HDM induced airway inflammation, was increased remarkably with HDM extract stimulation. Moreover, the levels of secreted clusterin determined in culture supernatants were significantly enhanced with HDM stimuli. To further investigate the role of clusterin in CCL20 secretion, we generated clusterin over-expressed BEAS-2B cells by transfection with adenovirus-containing clusterin gene. In response to HDM stimulation, the CCL20 secretion was remarkably suppressed in those clusterin over-expressed BEAS-2B cells.

Conclusion: In conclusion, it is speculated that the induction of over-expression of clusterin in bronchial epithelium may play a protective role in the airway inflammation.

429

Validation of a unique mobile environmental exposure chamber system for use in multiple locations in a multicenter allergy trial demonstrates accuracy and reproducibility in grass pollen aerosolization after mobile chamber disassembly, relocation and reassembly

Nandkeshore, H¹; Shields, K¹; Patel, P¹

¹Inflammax Research Inc., Mississauga, ON, Canada

Background: Controlled allergen challenge in fixed environmental exposure chambers (EECs) has proven benefits in single center

allergy trials. Our goal was to develop a mobile EEC system (mEEC) which was suitable for use in multiple geographically diverse locations in multicenter allergy trials. Towards this, we designed a transportable EEC system which demonstrates accuracy and precision in pollen aerosolization after mEEC disassembly, relocation and reassembly.

Method: Our proprietary mEEC design comprises inflatable shelter technologies built to clean room specifications and a patented mobile Environmental Control Unit (ECU) which controls allergen aerosolization characteristics. mEEC's were built to our design specifications in Europe, disassembled, relocated and reassembled at our headquarters in Toronto, Canada. Installation and Operational qualifications (IQ, OQ) were performed. Performance Qualification (PQ) testing in which grass pollen mix was aerosolized at 3500 ± 500 grains/m³ and acceptance criteria set out *a priori* were performed to demonstrate spatial and temporal airborne allergen uniformity across multiple samplers across the patient seating area. In phase I, ease and robustness of mEEC reassembly after shipping and relocation was tested as well as IQ, OQ, PQ and critical element failure. In phase II, the mEEC was disassembled and reassembled to test for IQ, OQ and PQ reproducibility.

Results: After disassembly, pack-up, and shipping of >5000 km, the mEEC was reassembled in Toronto, Canada. The mEEC inflation was complete in 1 h with ECU hook-up and configuration over 2 days. The system was robust with no damage sustained during travel and easily configurable. In Phase I, mEEC was shown to aerosolize grass pollen within specifications that demonstrated spatial and temporal uniformity across the patient seating area over triplicate testing, and simulation of critical element failure showed timely instrument switch and rectification. In Phase II, after disassembly-reassembly, PQ testing showed that mEEC performed identically and within specification.

Conclusion: The mEEC system testing demonstrates that the mEEC are suitable for use in multicenter allergy trials where multiple mEECs will be located in geographically diverse locales. This will allow for improved patient screening and

endpoint measurement where all patients are exposed to controlled and consistent allergen levels throughout the duration of the clinical trial.

430

Cohort profile: the Barwon Infant Study

Vuillermin, P^{1,2,3}; Carlin, J^{2,3}; Tang, M^{2,4,5}; Saffery, R^{2,5}; Allen, K^{2,4,5}; Ranganathan, S^{2,4,5}; Burgner, D²; Dwyer, T²; Ponsonby, A-L²; BIS Investigator Group

¹Barwon Health, Child Health Research Unit, Geelong, Vic., Australia; ²Murdoch Childrens Research Institute, The Royal Children's Hospital, Parkville, The University of Melbourne, Melbourne, Vic., Australia; ³Deakin University, Geelong, Vic., Australia; ⁴The Royal Childrens Hospital, Parkville, Vic., Australia; ⁵The University of Melbourne, Melbourne, Vic., Australia

Objectives: The Barwon Infant Study (BIS) is an unselected prebirth cohort study, with a unique array of longitudinally assembled biological samples, physiological measures and clinical outcomes. The objective of BIS is to investigate the interplay between the modern environment, the microbiome, the epigenome, abnormal immune development and the early life origins of eczema, food allergy, asthma and atherosclerosis. The initial hypotheses under investigation are that:

- 1 Reduced gut microbiota diversity at 1 month of age is associated with (a) persisting methylation (epigenetic silencing) of the interferon gamma (IFN γ) promoter of naïve T cells; (b) diminished IFN γ response capacity; and (c) increased risk of eczema and food allergy.
- 2 (a) Reduced IFN γ response capacity of naïve T cells during infancy, (b) higher incidence of lower respiratory tract infections, and (c) aeroallergen sensitisation, are associated with deterioration in lung function between 1 and 36 months of age.
- 3 Perinatal and early life factors, including an increased microbial burden, are associated with increased early markers of atherosclerosis development between 1 and 36 months of age.

Methods: BIS is located in the Barwon region of Victoria, Australia. Using an unselected sampling frame, women are recruited before 28 weeks of pregnancy (target $n = 1250$ over 3 years). Participants

are reviewed 6 monthly. In addition to extensive questionnaire data, biospecimen collection includes: stool (maternal at 36 weeks of pregnancy; infant at birth, 1, 6, 12, 24 and 36 months), and blood (maternal at 28 weeks of pregnancy; infant at birth, 6, 12 and 36 months). Physiological measurements include: lung function (via Multiple Breath Washout) at 1 month and 3 years, and aortic intima media thickness (via transabdominal ultrasound). Food allergy status is defined by formal challenge. A nested case-cohort design will be used to assess the biological hypotheses requiring resource intensive laboratory procedures.

Progress: Fieldwork commenced in June 2010. As of November 2012: there were 911 women enrolled (on target); 756 live born infants; among eligible infants the retention rate to 1 year was 246/293 (84%); biosamples were successfully collected on more than 90% of occasions for each timepoint/sample; and at the 1 month review 535/670 (80%) had undergone transabdominal ultrasound, and 404/670 (60%) had undergone lung function testing.

432

Study of immuno-physiological mechanism implicated in atopic march using an original mouse model of food and respiratory combined allergy

Bouchaud, G¹; Gourbeyre, P¹; Bihouée-Roussey, T²; Aubert, P³; Lair, D²; Denery-Papini, S¹; Neunlist, M²; Magnan, A²; Bodinier, M¹

¹UR 1268 BIA INRA, Equipe Allergie, Nantes, France;

²INSERM U915 IRS-UN, Institut du Thorax, Nantes, France; ³INSERM U913, IMAD, Nantes, France

Background: The increase prevalence of atopic disease has become a major challenge for allergists and public health authorities. The natural history of the atopic march including mechanism implicated and modifiable determinants are still very poorly understood. Information provides by clinical studies and mouse model can only generate hypotheses. Establishment of a new mouse model combining food and respiratory allergy define a way to analyse the atopic march by investigating immunological and physiological parameters.

Methods: Mice were sensitised to wheat allergens by intraperitoneal and oral administration and then exposed to house dust mite allergens in intranasal. Allergic reaction was monitored by measuring levels of IgE and histamine. Then, immune response was evaluated by cell number and cytokine production in lymphoid organs (spleen and thymus), gastro-intestinal tract (Peyer patches) and respiratory tract (lung and broncho-alveolar fluid). Physiological parameters were also analysed by exploring

paracellular and transcellular permeability as well as feces humidity in the gut and airway hyperresponsiveness in the lung.

Results: Our model revealed influence of combined allergy on the physiopathology of allergy. After food and respiratory allergen exposures, our data demonstrate a higher level of IgE specific to each allergen and histamine. Moreover, we observed an increase level of IL-4 and IL-17 accompanied by a decrease level of IL-10, IFN γ and TGF- β in Peyer patches. In parallel, we show higher paracellular and transcellular permeability in proximal colon and jejunum respectively. Surprisingly, airway parameters (hyper-responsiveness and inflammatory cells) exhibited no significant changes when compared to mice only exposed to respiratory allergens.

Conclusions: Our new mouse model displayed an increase of allergenic biomarkers including specific IgE and histamine levels, as well as Th2 and Th17 cytokines compared to a single allergy. In consistent with these results, we show a decrease of tolerance-related biomarkers especially Th1 and Treg cytokine suggesting interplay within immune allergic mechanism implicated in both allergies. Within this line, combined allergies induce stronger alteration of gut functions which contrasts with the absence of major changes on airway functions. Taken together, our results demonstrate a relationship in mechanism implicated in food and respiratory allergy in atopic march.

433

Correlation between serum total IgE and asthma severity in patients with allergic asthma in Spain

Dávila, I¹; Entrenas, LM²; Valero, A³; Herráez, L⁴

¹Hospital Universitario de Salamanca, Servicio de Inmunología, Salamanca, Spain; ²Hospital Universitario de Reina Sofía, Córdoba, Servicio de Neumología, Córdoba, Spain; ³Hospital Clínic de Barcelona, Unidad de Alergia, Barcelona, Spain; ⁴Departamento Médico, Novartis Farmacéutica, Barcelona, Spain

Background: Immunoglobulin E (IgE) has a key role in the pathogenesis of allergy. The close association between IgE, allergy, and asthma has been long recognised. Although the inflammatory pathways involving IgE are now better understood, its relationship to the asthma severity is still controversial. The aim of our study is to assess the possible association between serum concentration of total IgE and asthma severity.

Methods: An observational, retrospective, multicenter and national study was carried out in 63 Pneumology and Allergy centers. Data have been collected retrospectively on

all patients aged ≥ 18 , who have been diagnosed with persistent asthma (minimum a year of evolution) and positive skin prick-test or specific IgE (at least one aeroallergen). According to GEMA 2009 asthma was stratified by mild, moderate and severe. Patients with high IgE level not related to asthma, active smokers, ex-smokers (>10 pack/year) or ex-smokers (<2 years previous to the study) were excluded. The comparison between the three severity asthma groups of serum total IgE (logarithmic scale) was performed using univariate and multivariate analyses, adjusted for confounding variables (age, sex, atopy, smoking, onset of asthma, illness duration and severity of the disease according to GEMA). Furthermore, association of serum total IgE with FEV1, exacerbations or geographical site was evaluated.

Results: The study included 383 patients with a mean age of 43.0 ± 15.6 years, mainly men (58%) and 57.4% presented overweight/obesity. The disease had been made its debut earlier among patients with highest level of asthma severity, number of exacerbations and number of steroid treatment batches ($P < 0.05$, in all cases). The mean serum concentration of IgE (logarithmic scale) was similar between the three asthma severity groups (5.4 ± 1.1 UI/ml mild asthma [$n = 129$], 5.4 ± 1.0 UI/ml moderate asthma [$n = 82$] and 5.4 ± 1.3 UI/ml severe asthma [$n = 172$]). Asthma severity did not show statistical association to IgE levels after adjusting for confounding variables in univariate and multivariate analyses. Nevertheless, lower age and male sex ended up being statistically linked to highest IgE levels ($P < 0.05$). Finally, no associations of IgE with FEV1, exacerbations or geographical site were found.

Conclusion: Results from this study show there is no association between serum concentration of total IgE and asthma severity. However lower age and male sex were significantly connected to highest IgE levels.

435

Defective epithelial barrier function related to zonula occludens proteins of nasal mucosa in subjects with atopy

Yilmaz, O¹; Inan, S²; Pinar, E³; Turkeli, A¹; Turkoz, E²; Toprak Kanik, E¹; Yuksel, H¹

¹Department of Pediatric Allergy and Pulmonology, Celal Bayar University, Manisa, Turkey; ²Department of Histology and Embryology, Celal Bayar University, Manisa, Turkey; ³Izmir Ataturk Training and Research Hospital, ENT, Izmir, Turkey

Background: Most important proteins of the tight junctions, which are important

for epithelial barrier dysfunction in pathogenesis of allergic sensitisation due to their direct influence on antigen permeability, include occludin, claudin and junctional adhesion molecule (JAM). We aimed to investigate integrity of tight junctions via immunohistochemical determination of nasal mucosal occludin, claudin and JAM levels in atopic individuals.

Methods: This cross-sectional study enrolled 68 consecutive patients without allergic rhinitis symptoms who had nasal septum deviation surgery. Age and gender of were recorded and skin prick test (SPT) with major inhalant allergen extracts was performed according to EAACI guidelines. Mucosal biopsy specimens, taken from lower concha medial surface during nasal septum surgery, were immunostained with primary antibodies for occludin, claudin and JAM using indirect avidin-biotin peroxidase method and intensities were semi-quantified with H-score.

Results: Among patients enrolled, 14 had SPT positivity. Age and gender were similar between atopic and nonatopic patients ($P = 0.56$ and $P = 0.13$ respectively). Median occludin H-score was significantly lower in nasal mucosa of atopic than nonatopic patients (142.5 vs 290 respectively, $P < 0.001$). Median claudin H-score was 153 in atopic compared to 296 in nonatopic patients ($P < 0.001$). Moreover, nasal mucosal JAM was significantly different between the two groups (median H-scores 156 and 312 respectively, $P < 0.001$).

Conclusion: Expressions of occludin, claudin and JAM which are tight junction proteins of nasal mucosal epithelial cells are lower in atopic individuals suggesting a primary role of epithelial barrier dysfunction in development of atopic response. This finding carries the potential basis for new therapeutic modalities.

436

Visualisation of allergen uptake by dendritic cells

Ashjaei, K¹; Lengger, N¹; Bublin, M¹; Ellinger, I¹; Wagner, S¹; Hoffmann-Sommergruber, K¹

¹Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Background: Dendritic cells (DCs) are highly specialised antigen-presenting cells that regulate innate and adaptive immune responses and have been shown recently to play a key role in inducing T helper type 2 responses in allergic patients. Early events at the interface of allergens and DCs play a key role in downstream events leading to allergic reactions. Therefore, identifying mechanisms and receptors that are

involved in the initial recognition and uptake of allergen by DCs would lead to better understanding of the molecular basis of allergy and the rational design of novel intervention strategies. In this study we have studied allergen uptake by DCs, derived from allergic and nonallergic donors.

Method: Heparinised blood samples were obtained from grass pollen allergic and nonallergic donors and immature monocyte-derived dendritic cells (MoDCs) were generated as previously described. Purified rPhl p 5 and control allergens (sunflower LTP and transferrin) were labelled with Cy3, Alexa fluor 488 and FITC, respectively. On day 7, MoDCs were tested for surface binding (4°C) and internalization (37°C) of the allergens. Co-localisation of FcεRI, CD23 and Phl p 5 was also investigated. The cells were analyzed using fluorescence microscopy (Imager, ZI, ZEISS). For qualitative and quantitative assessment, Tissue-FAXS (Tissue-Genostic) and Tissue-Quest software was used.

Results and Conclusion: In this study supported by SFB F46-B19 (KHS) from the Austrian Science Fund, we have studied allergen uptake by DCs. The data demonstrated Phl p 5 binding and internalization by MoDCs from both, allergic and nonallergic individuals. In competitive uptake of mixed allergens (Phl p 5+LTP), MoDCs from allergic subjects showed preferential uptake of Phl p 5 as compared to LTP. Co-localisation experiments (CD23, FcεRI, Phl p 5) suggest that more than one mechanism facilitate the allergen uptake by MoDCs.

437

Potential antiallergic effect of pleuran (beta-glucan isolated from *Pleurotus ostreatus*)

Jesenak, M¹; Banovcin, P¹; Sanislo, L²; Rennerova, Z³; Majtan, J⁴

¹Department of Paediatrics, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia; ²Department of Clinical Immunology and Allergology, St. Elizabeth's Oncology Institute, Bratislava, Slovakia; ³Pneumo-Allergo Centrum s.r.o., Bratislava, Slovakia; ⁴Slovak Academy of Sciences, Institute of Zoology, Bratislava, Slovakia

Background: The prevalence of allergic diseases is steadily increasing. There is a search for complementary alternatives to standard antiallergic treatments. Beta-glucans are a group of biologically active polysaccharides of natural origin with a proven pleiotropic immunomodulation effect. Several studies suggest possible antiallergic therapeutic and preventive effects of beta-glucans. We studied the possible effects of pleuran on nonspecific markers

of allergy (total IgE, peripheral blood eosinophilia) and on the production of cytokines *in vitro*.

Method: In a double-blind, placebo-controlled study, we investigated a group of 175 children (aged 5.65 ± 2.39 years). Children were randomised into an active group (treated with pleuran in the form of syrup for 6 months) and a placebo group. During three visits (V0 – at the beginning, V1 – after 6 months, V3 – after 12 months) blood was sampled for the examination of the nonspecific allergic markers. In a subgroup of the subjects the sampling for stimulated *in vitro* cytokine production was performed.

Results: Whereas in the active group, the level of total IgE in serum remained stable across the whole study, in the placebo group the levels of total IgE increased gradually from V1 to V3. The eosinophilia in peripheral blood decreased significantly as a result of active treatment, but in the placebo group, we did not detect any changes of this parameter. In a subgroup of allergic patients, we studied the potential effect of pleuran *in vitro*. After 24 h of stimulation of whole blood with either pleuran or lipopolysaccharide (TLR agonists), we observed a significant increase of IL-10 and GM-CSF production and a decreased TNF-α production in the pleuran group vs the lipopolysaccharide group. Other cytokines did not show significant changes.

Conclusion: Our study provides relevant evidence of the potential antiallergic effect of pleuran. This supports the use of pleuran (beta-glucans) as a complementary treatment in allergic diseases. Our study opens new perspectives on the use of this widespread and popular group of natural substances. This effect needs further investigation.

438

Patient serum sensitivity profiles for allergens prevalent in Italy is geographically relevant

Swan, NJ¹; Hewings, SJ¹; Hutchings, JW¹; Mwange, JD¹; Skinner, MA¹

¹Allergy Therapeutics, R&D, Worthing, United Kingdom

Background: Suitable serum is required for adequate characterisation and standardisation of allergen SCIT products. Olive, Parietaria and Cypress species are more prevalent in Southern Europe though sensitised patients exist worldwide. This study compares the sensitivity profile of serum sourced within and outside Italy.

Method: Patients allergic to Olive, Parietaria and Cypress pollens were enrolled during the pollen season as they sought

treatment. Blood was taken prior to treatment and components separated. Individual patient serum was then tested quantitatively using total allergenicity titre and qualitatively using Western blotting of Olive, Parietaria and Cypress pollen extracts. The individual patient serum samples were analysed alongside a serum pool generated outside the region.

Results: Western blotting demonstrated a response in all patients to the relevant species, however it was noted that the patients react with differing intensities. In addition patients' serum reacted to different allergens or combinations of allergens. This variation in responses was also observed in the comparative total allergen titres with a range of dose response curves identified.

Conclusion: The study has shown diversity in patient sensitivity profiles for allergens.

439

Modification of the allergic response in patients undergoing autologous hematopoietic stem cell transplantation

Izquierdo Domínguez, A¹; Labrador Horrillo, M¹; Sala Cunill, A¹; Guilarte Clavero, M¹; Barba, P¹; Cardona Dahl, V¹

¹Hospital Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain

Background: During to the procedure of hematopoietic transplant, a modification and restructuring of the immune system occurs in the patient. This 'reset' of the immune system has been studied in different autoimmune diseases, but not in allergic conditions. The objective of this study was to evaluate whether allergic patients undergoing autologous hematopoietic stem cell transplantation (ASCT) showed changes in sensitisation and/or improved their allergic symptoms.

Method: Prospective observational study. Adult patients undergoing ASCT from May 2010 to March 2012 at Hospital Vall d'Hebron, Barcelona, Spain were invited to participate; patients were assessed by medical history, total IgE, specific IgE and component resolved diagnosis (ImmunoCAP & ImmunoISAC, ThermoFisherScientific, Sweden) before and 4–6 months after autotransplantation. Informed consent was obtained from patients and the protocol was approved by the ethics committee.

Results: Out of 28 patients included, only 6 (21%) showed some allergic sensitisation

pre-ASCT; in 5 (83%), specific IgE levels decreased or became negative and allergic symptoms disappeared post-ASCT. While there was a significant difference in serum total IgE between allergic [median 68.10, interquartile range (IQR) 12.5–850 KU/l] and non-allergic (median 2.91, IQR 2–85 KU/l) patients pre-ASCT ($P = 0.013$), this difference disappeared after the procedure.

Conclusion: In this pilot study, allergic sensitisation was diminished after ASCT, and this was in parallel to clinical improvement.

441

Allergic reaction in Japanese patients with obstructive sleep apnea syndrome (OSAS) and upper airway resistance syndrome (UARS)

Inaba, Y¹; Suzuki, Y¹; Tokano, H¹; Iwasaki, A¹; Tsunoda, A¹; Kitamura, K¹

¹Department of Otorhinolaryngology, Tokyo Medical and Dental University, Tokyo, Japan

Background: In most cases with OSAS, obstruction occurs in pharyngeal space. In contrast, obstruction remains unclear in some Japanese patients because of short length of facial anteroposterior diameter and they demonstrate low score of apnea-hypopnea index (AHI) on polysomnography. They might be diagnosed UARS because they experience sleep disorder and demonstrate lowered SpO₂ level during sleep. In the present study we analyzed allergic reaction in Japanese patients with OSAS and UARS and we studied if the allergic reaction could influence the clinical findings in patients with OSAS and UARS.

Method: The subjects were 24 Japanese patients with OSAS and/or UARS who undertook treatment including three cases of oral aid, six cases of medication, five cases of CPAP, and 10 cases of surgery. The data of allergic reaction, such as number of eosinophils, and serum levels of RIST and RAST were measured and their correlation with sleep disorder was evaluated.

Results: Elevated levels of specific antibodies against house dust and mite were seen in almost all groups.

Conclusion: These results suggested that elevated levels of specific antibodies against house dust and mite correlate sleep disorder

der regardless of the degree of AHI or lowered SpO₂ level.

442

Allergic rhinitis is associated with increased levels of regulatory T cells

Kaidashev, IP¹; Sakevich, V¹; DuBuske, LM²

¹Ukrainian Medical Stomatological Academy, Poltava, Ukraine; ²Immunology Research Institute of New England, Gardner, MA, United States

Background: CD4⁺CD25⁺ regulatory T cells (T reg), which regulate the functions of T cells, provide the directions for various types of immune responses. The mechanism of allergic rhinitis development is the imbalance among different populations of Th1/Th2 lymphocytes with Th2 cells prevalence. The natural regulatory T cells (nTreg) as the key immunoregulatory cells are able to suppress the Th1-dependent immune responses. The specific intracellular marker for identifying Tregs is known as transcription factor (FoxP3), which is also important for the development and functioning of these regulatory cells.

Method: A group of 45 persons (23 men, 22 women), age 19–65 years old, with allergic rhinitis was assessed. The levels of expression of CD4⁺, CD4⁺/25⁺, CD4⁺/25⁺/Foxp3 were determined in mononuclear suspensions of peripheral blood (Sorbent, Russia; eBioscience, USA).

Results: According to the results of immunogram, the levels of CD4⁺ did not significantly differ from healthy controls being $40.5 \pm 1.2\%$. There was a growth in the relative number of CD4⁺CD25⁺Foxp3⁺Treg ($4.66 \pm 0.38\%$) of total lymphocytes in comparison with healthy controls. To assess the imbalance of the immune system and establish the presence of statistical relationships between the immunological parameters of patients with allergic rhinitis vs healthy controls, correlation analysis using the Spearman's method was done, demonstrating a moderate positive correlation between the parameters: CD4⁺CD25⁺Foxp3 and CD4⁺ ($R = 0.43$; $P = 0.00$); CD4⁺CD25⁺Foxp3 and the number of eosinophils ($R = 0.35$; $P = 0.03$).

Conclusion: These derived data confirm the close relationship between the allergic inflammation and the numbers of regulatory T cells.

Poster Session 3

Clinical issues in autoimmunity

444

The possible role of *Helicobacter pylori* in primarily non gastrointestinal autoimmune and/or autoinflammatory processes

Szakos, E¹; Szontagh, E¹; Vamosi, I²; Vizi, J¹; Tamasi, K¹; Barkai, L¹

¹Borsod County University Hospital, University of Debrecen, Postgraduate Institute of Pediatrics, Miskolc, Hungary; ²Borsod County University Hospital, Clinical Laboratory, Miskolc, Hungary

Background: Microbiological agents are suspected launching the development of sterile inflammatory processes in genetically predisposed individuals by molecular mimicry. The authors tested possible etiological role of *Helicobacter pylori* (HP) in the case of patients with primarily non gastrointestinal autoimmune and/or autoinflammatory (sterile inflammatory) processes.

Patients and Methods: Authors followed up clinical and laboratory data of 165 individuals with sterile inflammatory diseases examined at Dermatology-immunologic outpatient's Department. They performed analysis of data collected by prospective and retrospective ways. Blood chemistry, autoantibody, *Helicobacter pylori* serology testing are also done. Upper gastrointestinal endoscopic procedures and HP immune histochemistry were performed in some cases with suspected active HP infection. Fifty control patients (32 girls, 18 boys) tested with HP serology presented gastrointestinal symptoms.

Results: One hundred and thirty (71 girls, 41 boys) of 165 patients aged 2–18 years, 52 of them (43 women, 9 men) aged 19–52 years. 40 of 165 patients (18 children: 9 boys, 9 girls, 22 adults: 6 male, 16 female) presented HP serologic positivity. Occurrence was 15.8% in children, (12.65 in girls, 21.45 in boys) 42.3% (37.7 in women, 66.6 in man) in adults with sterile inflammation. HP positivity in controls was: 32/50 64% (21/32 girls 65.6%, 11/18 boys 61.1%). 85 of sterile inflammatory processes occurred in 40 patients with HP positivity: arthritis-arthralgia 18, thyroiditis 16, idiopathic urticaria 8, alopecia areata 7, cutan vasculitis 5, psoriasis 5, habitual aphtha 5, vitiligo 4, Raynaud syndrome 3, habitual abortus 3, scleroderma 2, lichen sclerosus 2, coeliakia 2, granuloma anulare 1. Type I diabetes mellitus 1, acne conglom-

bata 1, obesity 1, rosacea 1. ENA was the most frequent autoantibody: 17/40 in cases with HP positivity, in 25/125 with HP negativity. Odds ratio: 1.875. Anti smRNP occurred: 2/40 and 20/125. Odds ratio: 0.31, anti double stranded DNA positivity 5/40 and 28/125, Odds ratio 0.55.

Conclusion: Higher frequency of HP positivity among male with sterile inflammation could be new information, waiting for confirmation. Likely linked occurrence of ENA and HP positivity in these patients with non polyserositic sterile inflammation arises the possible role of HP colonization in initiation of sterile inflammation. More studies are needed clarifying the role of HP in sterile inflammation.

450

Systemic lupus erythematosus-vasculitis – a national scientific research

Yakovliev, PH¹; Nikolov, K²; Baleva, M²; Shomov, G³; Kishkina-Takovska, N⁴

¹Consulting Room of Allergology, Stara Zagora, Bulgaria; ²Medical University, Sofia, Bulgaria; ³Medical University, Stara Zagora, Bulgaria; ⁴Consulting Room of Allergology, Blagoevgrad, Bulgaria

Background: We examined and monitored 56 patients with SLE vasculitis, at the age of 24–73 years, being treated in the hospital in Stara Zagora, Bulgaria, with duration of the illness from 1 to 17 years.

Method: From the *immunological parameters* we examined: IgG, M, A; C3 and C4 complement fractions – Radial immunodiffusion; antibodies to nuclear antigens DNA; Sm; RNP – with Counter electrophoresis; ANA and ANCA antibodies with Immunofluorescence method; anti-cardiolipin antibodies – with ELISA, RF – with Agglutination and ELISA, as well as CIC (circulating immune complexes), histones, crioglobulins; Ro and La.

Results: Most frequently observed clinical symptom in the monitored ills was joint affection, reported in 38 ills (67.9%), followed by skin lesions manifested by petechiae, palpable purpura, urticaria, nodules, bullae, ulcerations and others in 37 patients (66.1%). Kidney damage was also often observed, in 31 (55.4%) from the monitored patients, and high temperature was measured in 30 ills (54%). According to our data, mean values of immunoglobu-

lins in the serum of investigated patients with SLE vasculitis are higher than in healthy people, but statistically significant ($P < 0.001$) is only elevation of IgG and IgA. Elevated levels of IgG we found in 25 ills, and of IgA – in 27. Levels of IgM were not elevated in our patients. Our data shows that values of serum C3 and C4 in ills with lupus vasculitis are significantly lower than in healthy people ($P < 0.001$). In 42 of our patients anti-cardiolipin antibodies were above upper limit of reference range for immunoglobulin classes IgG and IgM and compared to the control group of healthy people there is a statistically significant difference ($P < 0.001$). In 15 of our patients with high acL titer we observed more often phlebothromboses, insults and infarctions, as well as skin vasculitis, as opposed to ills with low titer of these antibodies. Mean values of CIC for our patients with SLE vasculitis are higher from those in healthy people, and the difference is statistically significant ($P < 0.001$) according to PEG and Clq method.

Conclusion: In our opinion, determining the composition of CIC is important, especially in cases when ANA are not found in the serum of ills with SLE vasculitis. The majority of our patients with SLE vasculitis were with positive ANA (76%) and DNA (60%). DNA antibodies are important criteria for diagnosis of systemic lupus, accompanied with vasculitis.

451

Raynaud's Syndrome and vasculitis

Yakovliev, PH¹; Nikolov, K²; Baleva, M²; Shomov, G³; Kishkina-Takovska, N⁴

¹Consulting Room of Allergology, Stara Zagora, Bulgaria; ²Medical University, Sofia, Bulgaria; ³Medical University, Stara Zagora, Bulgaria; ⁴Consulting Room of Allergology, Blagoevgrad, Bulgaria

Background: Totally 65 ills have been examined: 35 had only Raynaud's syndrome (group I), and other 30 were with Raynaud's syndrome included in another disease, respectively: with SLE (16), with scleroderma (10) and with mixed connective tissue disease (4) – group II. The patients that we examined were at the age of 18–73 years and duration of the illness from 6 months to 12 years.

Method: Distributions of positive ANA, as well as their specificity differ between the two groups. It is important to note that positive ANA are suggestive for the need of further examinations.

Results: In the first group are prevalent the negative results and those with low titers – 70% from the ill, and the antibodies are mainly to extractable nuclear antigens (EHA) – anticentromere, anti-RNP, anti-Scl-70. On the opposite – 80% from the patients from group II had high titers of ANA, and their characteristic is heterogeneous – half of them are anti-DNA, and the other half – anti-EHA. Major clinical symptoms in ill with Raynaud's syndrome are: bruising on the fingers (80) and formication and tingling sensation of the affected limbs (97%). With livedo reticularis were 51% of the ill from group I. Significant diagnostic features are ulcerations and necroses on distal phalanges, observed in 31% of the examined patients with Raynaud's syndrome. In group II symptoms such as arthritis/arthritis (86%), myalgia and bruising on fingers (66%), photosensitivity, distinctive rash, habitual abortions and other were prevalent.

Conclusion: Major clinical symptoms of Raynaud's disease are:

- Vascular attacks, induced by chill and excitement;
- Localisation of vascular attacks is symmetric;
- Arterial pulsation of all reachable accesses is normal;
- Gangrene of the skin on distal parts of the limbs;
- Vasospasm is observed during a period of at least 2 years;

The main laboratory and instrumental parameters are:

- In our patients, angiography of vessels of distal parts of the limbs shows areas of irregular stenosis, partial or total obliteration and lack of additional capillary anastomoses/72%;
- By capillaroscopy of the nail bed or bulbar conjunctiva we observed alterations of the shape and permeability of capillaries and microvessels/56%, reduction in their number or dilatation/32%. Severity of the changes corresponds to microcirculatory disorders.

453

Anti-PM/Scl and hypomyopathic dermatomyositis: a novel association

Allegro, E¹; Fassio, F¹; Salvati, G¹; De Giorgi, G¹; Troilo, A¹; Parronchi, P¹; Maggi, E¹

¹Immunology and Cell Therapies Unit, AOU Careggi, Florence, Italy

Case Report: We report the case of a 73 years old man, who referred to our

Unit for infiltrated erythematous skin lesions localised on the face, neck and proximal limbs.

Routine blood tests demonstrated increased values of CPK, myoglobine and ALT. Search for autoantibodies revealed the presence of antinuclear antibodies (ANA 1:320 speckled) and anti-PM/Scl. The patient subsequently underwent further investigations for connective tissue disease, even if he didn't complain for any other symptom at the time of consultation and physical examination, except for the above mentioned skin lesions, was unremarkable.

Physical tests, electromiography and muscle NMR didn't show any sign of muscle involvement. Arterial blood gas analysis, pulmonary function tests, including DLCO, and chest HRCT didn't show any sign of interstitial lung disease. Renal function was normal with no proteinuria.

We therefore performed skin biopsy which showed lichenoid tissue reaction consisting of vacuolization of basal layer cells and oedema of papillary dermis, associated with dilatation of superficial vessels and dermal infiltration predominantly consisting of lymphocytes and minimal numbers of neutrophils and eosinophils.

Being these findings consistent with a diagnosis of dermatomyositis (DM), and excluding any muscle involvement, we concluded for a diagnosis of hypomyopathic DM. This clinical entity is a rare subset of dermatomyositis which is characterised by DM-specific skin disease and subclinical evidence of myositis on laboratory, electrophysiologic and/or radiologic evaluation.

Anti-PM/Scl are autoantibodies which can be found in polymyositis/systemic sclerosis overlap syndromes. To date, no other cases of hypomyopathic dermatomyositis have been reported in association with anti-PM/Scl. The patient is currently being followed up as he may develop myositis and/or systemic sclerosis manifestations in the future.

455

Eosinophilia with organ involvement in two siblings

Lishchuk-Yakymovych, K¹; Pukalyak, R¹; DuBuske, I²; DuBuske, L²

¹Department of Clinical Immunology and Allergology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine; ²Immunology Research Institute of New England, Gardner, MA, United States

Background: Churg-Strauss syndrome (CSS) is a rare, autoimmune small and medium vessel vasculitis, accompanied by asthma and involves mainly the blood vessels of the lungs, and peripheral nerves,

skin, kidneys, and heart with marked blood eosinophilia.

Method: Two siblings who suffered from marked eosinophilia with organ involvement are described.

Results: One sibling experienced erythema multiforme, cervical lymphadenopathy, peripheral neuropathy, arthritis with eosinophilia (1.8 cells/ μ l) following bronchial asthma, was pANCA-positive and was diagnosed with Churg-Strauss syndrome (CSS) according to the criteria of the American College of Rheumatology. Glucocorticoid treatment with an initial daily dose of 40 mg of prednisone relieved eosinophilia, while combination of glucocorticoid with azathioprine using a daily dose of 100 mg relieved organ involvement symptoms. Another sibling, who suffered from severe asthma with persistent polyarthritis and eosinophilia (5.2 cells/ μ l), was cANCA-positive but did not fulfil the widely used criteria for CSS or hypereosinophilic syndrome (HES). This sibling was treated with 60 mg of prednisone daily dose with minimal reduction of eosinophilia.

Conclusion: Increased C-reactive protein and decreased anti-ET IgM, anti-ET IgG predicted a lethal outcome in 7 HD patients. Four years of monitoring chronic kidney disease hemodialysis patients showed a 4.6 fold increase in C-reactive protein ($P < 0.001$) but no change in indicators of humoral anti-endotoxin immunity.

457

Increased peripheral T_H17 Cells in SAPHO syndrome: a novel target for treatment?

Firinu, D¹; Lorrai, MM¹; Barca, MP¹; Peralta, MM¹; Mura, MN¹; Perra, S²; Cabras, S²; Manconi, PE¹; Del Giacco, SR¹

¹Department of Medical Sciences 'M. Aresu', Unit of Internal Medicine, Allergy and Clinical Immunology, University of Cagliari, Monserrato, Italy; ²University of Cagliari, Monserrato, Italy

Background: SAPHO syndrome, characterised by a variable combination of Synovitis, Acne, Pustulosis, Hyperostosis and Osteitis, is a rare, often unrecognised disease with prominent inflammatory cutaneous and articular features. Recent evidences lead to consider SAPHO in the spectrum of Auto-Inflammatory Diseases (AID). Treatments with steroids, NSAIDS or TNF- α antagonists have been effectively employed. In humans, a key role of IL-1 β as a pro-inflammatory regulator in the priming and differentiation of T_H17 lineage and the central role of IL-1 β hypersecretion in AID is well established. In some AID IL-1 β has been implicated to lead towards a severe systemic inflammatory

syndrome, possibly mediated by a T_H17 skewed phenotype. The inflammatory $P2X_7$ - $IL1\beta$ axis has been previously shown to be dysregulated in SAPHO syndrome.

Method: We checked if the $IL-1\beta$ derangement observed in SAPHO syndrome could affect the peripheral T_H1 , T_H2 or T_H17 frequency, in comparison with groups of healthy individuals and rheumatic disease controls, which were matched for sex, age, disease duration and treatment status.

Results: T_H17 levels for the two groups of SAPHO and not-SAPHO patients are significantly different ($P = 0.009$). We also studied the interaction between the variables SAPHO and treatment by means of a Fisher's exact test which showed no significative dependence. T_H1 and T_H2 levels for the two groups of SAPHO and not-SAPH-O patients are not significantly different.

SAPHO patients showed significantly increased numbers of $IL-17$ producing T-cells by flow-cytometry after PMA/ionomycin stimulation as compared to healthy controls. The frequency of T_H17 cells between SAPHO patients and rheumatic controls, matched for sex, age, disease status and treated with the same drug (Methotrexate plus Adalimumab) was significantly increased, irrespectively of disease status or treatment. We observed no correlation between T_H1 or T_H2 and T_H17 .

Conclusion: Our results suggest that activation of T_H17 axis, but not of T_H1 and T_H2 , is characteristic of SAPHO syndrome with protracted course, and that this finding is independent from remission status and/or from concurrent treatment with DMARDs or anti-TNF- α . Our preliminary data shed light on the possible involvement of T_H17 cells in the immunopathogenesis of SAPHO, irrespectively from the patient's disease status.

Targeting $IL-1\beta$ or $IL-17$ blockade may be considered in SAPHO patients on the basis of these results.

459

Patient with three years of papular lesions evolution

EGÜEZ HIDALGO, MF¹; De Luque Piñana, V¹; Botello Borrego, MD¹; Cabeza Rodríguez, N¹; Guardia Martínez, P¹
¹Allergy and Clinical Immunology Department, Virgen Macarena University Hospital, Seville, Spain

Background: Dermatitis herpetiformis is a chronic autoimmune disease that causes a rash pruritic vesicular papulo predominantly in extensor surfaces and characterised histologically by papillary microabscesses of neutrophils.

It is associated with gluten sensitive enteropathy and resolves after introducing a diet free thereof. It can occur at any age,

with a peak in the third decade. It is more common in males.

Method: A 72 year old male, ex-smoker, occasional drinker, diabetes mellitus.

Derived for 3 years of infiltrated papular lesions on elbow extension area, arms and face a few hours earlier with residual hyperpigmentation and lichenification with intense itching. Originally relate to making the gliclazide (sulfonylurea), however when removing limbs injuries persist. No alterations in bowel concerns. He had been treated as an atopic dermatitis.

Also referred sneezing clinic, no seasonal predominance hydrorrhea and unrelated to any trigger also associated with hyposmia.

Physical examination: Lichenified lesions on the back of his elbows.

Results: Basic blood analysis: 270 eosinophils/ml, Biochemistry: glucose 148 mg/dl, triglycerides 188 mg/dl, glycosylated hemoglobin 7.2. Urine: normal, PSA: normal.

Skin tests to aeroallergens, wheat, rye and barley: Negative

Epicutaneous test (true test): Negative

Total IgE: 267 U/l, specific IgE to aeroallergens: negative.

Transglutaminase IgA antibodies 112 U/l.

Skin biopsy with direct immunofluorescence study: granular deposits of papillary dermis at dermoepidermal junction and IgA, complement C3 and fibrinogen positive findings consistent with dermatitis herpetiformis.

Conclusion: We report a patient with injuries described as compatible with this condition over 3 years had been treated as an atopic dermatitis. Obviously this is a disease in which in their differential diagnoses include scabies, atopic eczema, contact eczema, and other autoimmune diseases such as bullous dermatosis linear IgA bullous pemphigoid, so it is important that we know a wide range of dermatological pathology related to an allergic or autoimmune mechanism, in which we can make differential diagnoses, which can sometimes go unnoticed as in our case as the patient had no digestive symptoms. As we reviewed the literature on the clinical presentation can be variable and associated digestive symptoms.

461

Association of KIR receptors polymorphism and MHC-class I alleles in Colombian Caribbean patients with lupus nephritis class IV

GARAVITO, G¹; OLIER, D¹; AROCA, G^{1,2,3}; IGLESIAS-GAMARRA, A⁴; GARCÍA, R²; EGEA, E^{5,6}
¹Universidad del Norte, Barranquilla, Colombia; ²Clinica de la Costa, Barranquilla, Colombia; ³Universidad Simón Bolívar, Barranquilla, Colombia; ⁴Universidad Nacional de Colombia, Bogotá, Colombia; ⁵Universidad del Norte, División Ciencias de la Salud, Barranquilla, Colombia; ⁶CliniAlergia, Unidad de Alergia, Barranquilla, Colombia

Background: Lupus nephritis (LN) is the most frequent complication in patients with SLE. To date it not has been defined any specific polymorphisms or haplotypes of KIR and MHC-Class I system as a marker of protection or susceptibility in admixed latin ethnic population. Given that genetic load of each ethnic group is very important in diseases association we designed this study in patients expressing the lupus nephritis class IV endophenotype in order to characterise an association between these genetic markers and the presence of this severe morbidity.

Method: This was a case-control study approved by the ethics committee of the University of North involving 50 individuals with class IV LN and 100 normal control subjects, none of them related familiarly and all residents on the same area Genotyping of KIR alleles was done by PCR-SSP and oligotipificación of HLA-B and HLA-C alleles by Luminex technology (HLA-SSO). Statistical analysis was done using SPSSv19 and Arlequin v3.11.

Results: The most frequent inhibitory KIR alleles were 2DL4 (100%), 2DL1 (98%) and 3DL2 (92%). Activators KIR alleles expressed were 2DS4 del (74%), 2DS4ins (46%) and 2DS1 (44%). Only statistical association was found as predisposing factors for alleles KIR3DL1 ($P = 0.001$) and KIR3DL2 ($P = 0.0001$). The activator alleles KIR3DL3 ($P = 0.0001$) and KIR2DS1 ($P = 0.0001$), KIR2DS2 ($P = 0.0001$), KIR2DS3 ($P = 0.0001$), KIR2DS4del ($P = 0.003$), KIR2DS4ins ($P = 0.0001$) and KIR2DS5 ($P = 0.0001$) were showing as protective factors. The HLA alleles more expressed were HLA-B*07 (30%), HLA-B*35 (30%), HLA-B*08 (20%), HLA-C*07 (60%) and HLA-C*04 (30%). Association as predisposing factors for developing class IV LN, was only found for the HLA-B*07 ($P = 0.002$) and HLA-C*07 ($P = 0.020$). The haplotypes with higher expression were HLA-B*07/HLA-C*07 (14%), HLA-B*35/HLA-C*04 (9%), HLA-B*08/HLA-C*07 (9%), HLA-B*40/HLA-C*03 (8%) and HLA-B*15/HLA-C*02 (6%) A protective factors was associated with the haplotypes

HLA-B*07/C*07 ($P = 0.0001$), HLA-B*08/C*07 ($P = 0.010$) and HLA-B*35/C*07 ($P = 0.030$).

Conclusion: This is the first study in SLE latin American patients done with these

genetic markers in search an association between those markers and the endophenotype class IV LN. The results suggest that These genetic markers could be used as predictive markers for this severe SLE

morbidity. More study could be performed for the validation of this results.

Poster Session 4

New developments in allergen-specific immunotherapy

462

Validation of a monoclonal antibody-based method for measuring olive pollen major allergen, Ole e 1

Arilla, C¹; Ibarrola, I¹; Brena, S¹; Santos, M¹; Zamarreño, J¹; Martínez, A¹; Asturias, JA¹

¹Bial Aristegui, R&D, Zamudio, Spain

Background: We developed a sensitive and specific two-site sandwich ELISA for the quantification of Ole e 1. It represents an important tool for quality control of allergen preparations from olive tree pollen intended for clinical use. The aim of this study was to demonstrate that the method is valid for the quantification of Ole e 1 in extracts according the 'Guideline on Allergen Products: Production and Quality Issues' (CHMP/BWP/304831/2007) and for the use in verifying the batch to batch consistency.

Method: The assay was based on 5A3 mAb as capture and biotinylated nOle e 1 specific rabbit polyclonal antibody as detector. The in-house reference extract from *Olea europaea* was analyzed and its allergen content was calculated using purified Ole e 1 as standard for the validation. The studied parameters were repeatability, intermediate precision, accuracy, limit of quantification and robustness. Ole e 1 content was also determined in extracts obtained from different pollen suppliers.

Results: The assay met the repeatability requirement since the CV for the percentages of Ole e 1 was 4.6%. None of the variables had a statistically significant effect on the test result, with a 95% confidence interval (minimum *P*-value 0.176). It fulfilled the requirement of accuracy since the $t_{exp} = 0.828$ calculated for the recovery test was less than the t_{tab} . The limit of quantification was determined in 9.8 ng/ml for the extract and fulfilled the requirements of precision (CV = 14.8%) and accuracy (relative error = 3.9%). The technique was robust for the effect of temperature and incubation time, so that the method complied with the specifications established for its use. Ole e 1 content among six batches of *Olea* pollen extracts showed good consistency with a coefficient variation of less than 25%.

Conclusion: The validated ELISA complements the biological standardisation of

different manufacturing batches by evaluating the major allergen in mass units.

463

Identification of relevant allergens by mass spectrometry: a new tool for standardisation of allergen preparations

Augustin, S¹; Reese, G¹; Klysner, S¹; Nandy, A¹

¹Allergopharma GmbH & Co. KG, Reinbek, Germany

Background: Traditionally, *in vitro* allergen standardisation has been conducted mainly by IgE-based assays. However, these tests are not appropriate to demonstrate the presence of all relevant allergens in a given allergen preparation. Assays based on allergen-specific animal antibodies can overcome this deficiency, but are dependent on the availability of these antibodies. As an alternative, mass spectrometry (MS) was tested for its applicability to identify allergens in various complex allergen preparations.

Methods: MS was applied for identification of relevant allergens in different allergen preparations from mites and pollens (derived from trees, grasses, cereals, and weeds). All investigated preparations were manufactured by Allergopharma. Analyses were conducted with production batches of allergen extracts used for allergoid production and with production batches of aluminium hydroxide-adsorbed extracts. In parallel current in house references (IHRs) were analysed. First, samples were subjected to enzymatic digestion. Resulting peptides were separated by liquid chromatography and subsequently analysed by tandem MS (LC-MS/MS). Allergen identification was performed by searching an individually designed allergen database containing all allergen sequences known from the analysed species.

Results: All current IHRs, three batches of each allergen extract and of each aluminium hydroxide-adsorbed allergen extract were analysed. The relevant allergens were identified in all samples by detection of peptides covering large parts of the respective proteins, leading to statistically significant protein scores. This included the allergens Der p 1, Der p 2, Der f 1, Der f 2, Bet v 1, Aln g 1, Cor a 1, Fag s 1, Ole e 1, Phl p 1, Phl p 5, Tri a 5, Sec c 5, Hor v

5, Ave s 5, Amb a 1, Art v 1, Par o 1, Par o 2, and Pla l 1. Furthermore, the majority of minor allergens known from the individual species were successfully identified.

Conclusions: Relevant allergens were identified reproducibly by MS in all analysed allergen preparations. These results demonstrate the quality of the investigated preparations and document that MS is a suitable technique to fulfil the demands of the current regulatory guidelines regarding identification of relevant allergens in pharmaceutical allergen preparations. Furthermore our data show that MS is applicable for comprehensive protein profiling of heterogeneous allergen preparations, provided protein sequence information is available.

466

Tyrosine as a biodegradable depot adjuvant for use in immunotherapy

Bullimore, AD¹; Hewings, SJ¹; Skinner, MA¹

¹Allergy Therapeutics, R&D, Worthing, United Kingdom

Background: An adjuvant is a material added to vaccine preparations to boost clinical efficacy. An ideal adjuvant should increase efficacy by prolonging exposure and/or boosting appropriate immune pathways. An adjuvant should not stimulate inappropriate immune mechanisms and should have minimal side effects. Additionally there should be efficient and complete elimination from the body after metabolism. The benefits of a tyrosine adjuvant platform have been investigated.

Methods: Data regarding the *in vivo* retention and elimination times of tyrosine when used as a depot adjuvant has been generated. In addition, specific IgG1 and IgG2 have been measured as indicators of whether Tyrosine can favourably balance the immune response.

Results: Candidate allergy therapies have been evaluated to consider the benefits of using the natural product tyrosine as a depot adjuvant in subcutaneous immunotherapy (SCIT). Tyrosine is naturally metabolised and the pharmacokinetics of tyrosine show that tyrosine has a half-life at the injection site of 48 h; this is a particular benefit for allergy SCIT, a traditionally long course treatment, minimising the need for accumulation of non-biodegrad-

able adjuvant. Data suggests that the speed of this elimination compared to alternative adjuvants leads to less likelihood of persistent nodule formation and injection site irritation. We have demonstrated that tyrosine can act as an adjuvant to induce increased IgG1 and IgG2 antibody production when the tyrosine is adsorbed to allergen preparations. Using specific IgG1 and IgG2 as a measure of response, it has been identified that tyrosine promotes re-balancing of the immune reaction. *In vivo* studies indicate a stronger Th1 inducing effect compared with that observed with alternative adjuvants and additionally suggests no IgE stimulation consistent with a Th2 response.

Conclusion: Tyrosine elimination and removal rates are greater than alternative adjuvants and tyrosine is effectively dispersed from the injection site, minimising the risk of injection site irritation. Stimulation of IgG1 and IgG2 is indicative of a Th1 response and, coupled with a lack of IgE induction, leads to the conclusion that tyrosine can be considered an ideal depot adjuvant. These findings form the basis for using tyrosine as a SCIT platform applicable for treatment of any allergy.

470

Satisfaction of patients with moderate/severe allergic rhino conjunctivitis treated with specific immunotherapy as oral lyophilisate under routine clinical practice conditions

Chivato, T¹; De la Torre, F²; Martínez, E²; OPTIMAL Study

¹School of Medicine, Boadilla (Madrid), Spain; ²ALK, Madrid, Spain

Background: There are two therapeutic options for allergic rhino conjunctivitis (AR). One is symptomatic treatment using drugs and the other allergen-specific immunotherapy. Furthermore, between the different routes of administration currently used in AR treatment (subcutaneous and sublingual).

Objective: To identify the expectations, clinical management and satisfaction of patients with moderate/severe AR treated with specific immunotherapy as oral lyophilisate.

Methods: A non-interventional, observational, multi-center, open-label study. Adult patients with a confirmed diagnosis of moderate/severe grass pollen-induced AR and treated with oral lyophilisate SIT between June 2010 and April 2011. Number of subjects planned and analyzed: 161 planned, 131 enrolled, 131 completed, 2 not evaluable and 129 analyzed. Age:

mean: 33.95, DT: 12.9. Sex: Male: 60 (46.5%) Female: 69 (53.5%).

Results: The expected result of the vaccine for each patient varies by AR type such that 2/3 of patients with a moderate/severe AR expect to 'recover completely' whereas those patients with a mild AR are less 'demanding'. Regarding the degree of satisfaction with the tablet-based vaccine, as assessed using horizontal and graduated visual analogue scales, should be noted: Symptoms remission and perceived efficacy: ranging between 72.3/100 and 77.4/100; Occurrence of side-effects: ranging between 67.9/100 and 74.8/100; Vaccine cost and availability is lower than above, ranging between 33.2/100 and 42.7/100; Comfort and ease of taking the vaccine: ranging between 82.4/100 and 89.1/100 and the overall degree of patient satisfaction is high, ranging between 77.1/100 and 79.9/100. The level of treatment adherence presented by the study sample for the tablet-based vaccine is either high or very high (more than 80% in the worst group). The degree of patient satisfaction does not differ significantly on the basis of the patient's expectations.

Safety results: Oral lyophilisate was perfectly tolerated. All adverse events (AE) registered were mild/moderate and no serious AEs were registered.

Conclusions: It is feasible to initially treat the interpretation of the relationship between patient satisfaction during treatment with SIT in tablet form, the understanding/expectations of SIT in general and the clinical management/amount of information received from the attending physician in a two-way manner between each element of this trial.

471

Characterisation of Parietaria allergoids with physicochemical techniques

de Bruijn, J¹; van den Hout, R¹; Cordewener, J²; America, T²; Luykx, D¹

¹HAL Allergy BV, Development, Leiden, The Netherlands; ²Plant Research International, Wageningen, The Netherlands

Background: Parietaria allergoids are drug substances for the Parietaria allergy vaccine. Until now these allergoids have been poorly characterised due to their complexity. Nevertheless, a better characterisation is needed. This includes identification of the relevant allergens and determination of the degree of polymerisation and cross-linking. Several physicochemical techniques were tested for their suitability to analyse these characteristics in the allergoid and corresponding allergen extract: Mass Spectrometry, SDS-PAGE, HPLC-SEC, lysine

determination and fluorescence spectroscopy.

Method: Allergoids: Allergen extracts from *Parietaria judaica* and *Parietaria officinales* pollen were treated with glutaraldehyde to obtain Parietaria allergoids.

MS: Preparation of tryptic digests and peptide separation via nano-LC before electrospray ionisation. Ionised peptides were fragmented revealing sequences.

SDS-PAGE: 10–20% gels with Coomassie Blue staining and reduced samples.

HPLC: A GF250 SEC column was used combined with UV-detection.

Lysine determination: Samples were hydrolysed into amino acids followed by free lysine determination with HPLC.

Fluorescence: Emission spectra were recorded from 290 to 400 nm, with excitation at 280 nm.

Results: Relevant Parietaria allergens Par j 1 and Par j 2 were identified in the Parietaria allergoids with MS. SDS-PAGE showed for the allergoids the formation of various high molecular weight molecules including masses ≥ 250 kDa. HPLC-SEC showed for the allergoids the formation of molecules ≥ 670 kDa. For the Parietaria extracts a minority of HPLC peaks were corresponding to proteins with a molecular mass >44 kDa (11%). For the corresponding allergoids the majority of peaks (52%) were corresponding to proteins with a molecular mass >44 kDa. Determination of free lysines showed that the majority of the lysines were modified in the Parietaria allergoids (76%). Fluorescence intensities obtained for the allergoids were very low indicating quenching.

Conclusion: Applying a combination of physicochemical techniques was shown to be a suitable approach to characterise the Parietaria allergoids well: Identification of the relevant allergens and determination of the degree of polymerisation and cross-linking were accomplished. Fluorescence spectroscopy appeared to be not suitable to analyse the protein structures of the Parietaria allergoids.

472

Development of a novel mite allergoid product for use in immunotherapy

Depreux, N¹; Jurgens, Y¹; Basagana, M¹; Roger, A¹; Garcia Cadena, G²; Bullimore, AD³; Skinner, MA³

¹H.U. Germans Trias Pujol, Unitat Allèrgia, Badalona, Spain; ²Allergy Therapeutics Iberica, Barcelona, Spain; ³Allergy Therapeutics, R&D, Worthing, United Kingdom

Background: *Dermatophagoides pteronyssinus* is recognised as one of the main causative agents of allergic rhinitis and allergic asthma in Europe and therefore there is a need for efficacious immunotherapy. A

tyrosine adsorbed, modified-allergen product has been developed for treatment of perennial mite allergy. The product has been standardised to meet a dose regime consistent with patient convenience and a prospective observational clinical study has been performed.

Methods: The product was designed with ICH (International Conference on Harmonisation) recognised Quality by Design (QbD) principles published. A prospective observational study of safety, tolerability and short-term effectiveness assessed by nasal provocation test and *in vitro* immunological changes is being conducted.

Adult patients (18–65 years) with a history of house-dust mite induced allergic

rhinitis/rhinoconjunctivitis with or without clinically stable mild or moderate asthma, were treated with subcutaneous immunotherapy containing 3000 SU/ml of modified *Dermatophagoides pteronyssinus* allergen extract. Tolerability and safety during dose escalation of 0.05, 0.1, 0.3 and 0.5 ml administered at 7 days interval were assessed. Nasal provocation data were collected 1 week before starting the treatment and 1 week after reaching maintenance dose. Flow-cytometry was used to determine cytokine activity 1 week before starting the treatment and 1 week after reaching maintenance dose.

Results: Fourteen patients were included: six females and eight males, average age of

37 (range 25–62). All patients followed the dose escalation schedule as it was originally prescribed. No local or systemic reactions occurred in patients during the dose escalation phase. Data on short term effectiveness (nasal provocation test and flow cytometry) will be presented in the future.

Conclusion: A novel product to treat allergic respiratory patients with modified *D. pteronyssinus* allergen preparation has been developed according to QbD principles. Adult patients receiving this *D. pteronyssinus* vaccine showed a good tolerability during the initiation dose escalation phase that permitted the completion of the dosage regime schedule as it was originally prescribed.

Poster Session 5

Drug allergy: diagnosis

474

Diagnosis in betalaktam allergy; comparison between commercial reagent kit and classical method

Kalpakioglu, AF¹; Kavut, AB²; Kalkan, IK¹

¹Immunology and Allergic Diseases, Kirikkale University, Kirikkale, Turkey; ²Immunology and Allergy Clinic, Erzurum State Training and Research Hospital, Erzurum, Turkey

Background: Skin testing represents the first-line method for diagnosing betalactam hypersensitivity. Although various studies have been performed with major and minor determinants of benzylpenicillin, in subjects with suspected betalactam allergy, relatively few comparable reports exist. Our aim was to evaluate the commercial kit of poly-l-lysine (PPL) and minor determinant mixture (MDM), to compare with the classical method using commercial drugs, and to test the results in potency, sensitivity, and specificity, as well as applicability.

Method: All patients >16 years old referred for a compatible history of allergic reaction to betalactams were included. Skin tests (prick/intradermal) were performed with DAP reagents including PPL and MDM (Diater, Madrid, Spain), followed by penicillin G, amoxicillin, aminocillin, and the culprit betalactam. If skin tests were negative, a single-blind oral provocation test (OPT) was performed with commercial penicillins.

Results: A total of 205 subjects were assessed, of whom 153 were patients with suspected betalactam allergy. 71.2% were female, with a median age of 37.69 ± 13.46 years. Overall test positivity was found in 34 (22.2%) patients; skin tests with DAP in 12 (10.3%), and with commercial drugs in 15 (9.8%). Three patients displayed a positive reaction to both PPL and MDM, while nine patients had a positive reaction to either PPL or MDM alone, using DAP. Fifteen patients (9.8%) developed positive reactions in OPT, with no significant difference between the species. Eight patients in whom a negative result was obtained with the commercial reagents in skin tests had a positive reaction to oral challenge. Sensitivity and specificity of skin tests using commercial reagent kit and commercial drugs are 33.3%–13.3%, and 96%–94.5%, respectively. Moreover, positive and nega-

tive predictive value of both tests were 44.4%–18.2%, and 94%–92.2%, respectively. The majority of the systemic reactions was immediate type and mainly belonged to skin.

Conclusion: Our results indicate that the commercial reagent kit is a reliable tool for the diagnosis of betalactam allergy, with high specificity and negative predictive value. This may be a safe alternative and a practical option in adult patients, where skin tests with commercial drugs are difficult to perform and dangerous. However, both tests can be preferred in exclusion of betalactam allergy, rather than the diagnosis.

475

Estimating of tryptase and eosinophilic cationic protein level in saline during provocative test with local anesthetics and dental materials: principals and advantages

Lazarenko, LL¹

¹Pavlov State Medical University, Saint-Petersburg, Russia

Background: The most reliable markers of allergic inflammation of the type I are tryptase and eosinophilic cationic protein (ECP). Tryptase – a mediator of mast cells, it mediates immediate hypersensitivity. ECP is granular protein of eosinophils, which is associated with chronic allergic inflammation.

Method: Provocative test with determination of tryptase and ECP in saliva was performed. Examined saliva samples from 28 patients with indications of intolerance to local anesthetics (group A – 8 men and 4 women), and prosthetic materials (group B – 5 men and 11 women). Determine the initial level of tryptase and ECP and its contents at 2 h after the application of a local anesthetic or a prosthetic material in the mouth. Studies carried out on the equipment ImmunoCAP100 E and reagents ImmunoCAP. Exclusion criteria were the presence of systemic mastocytosis and elevated baseline level of neurotransmitters in the saliva.

Results: Tryptase levels in healthy donors were 3.8 ± 2.1 mg/l, ECP – 5.9 ± 3.4 mg/l.

In group A, the average level of tryptase before provocation was 5.5 ± 1.1 mg/l, after provocation – 8.1 ± 1.45 mg/l. Positive provocative test for lidocaine for tryptase was detected in one patient (basal tryptase 4.4 mg/l, after – 12.4 mg/l). In the case history – introduction of lidocaine cause the angioedema. Another patient increased the level of ECP after mepivacaine provocation (before – 6.2 mg/l, after – 29.3 mg/l). In the case history – makulopapular rash and bronchospasm after 3.5 h treatment.

In group B the value of tryptase and ECP before and after challenge were not significantly different from baseline (tryptase in saliva – before – 5.9 ± 1.5 mg/l, after – 6.4 mg/l; ECP – before – 6.9 ± 1.3 mg/l, after – 9.7 ± 2.2 mg/l). Provocative tests were positive in three patients: one nickel to ECP (before – 6.4 mg/l, after – 25.6 mg/l), the other on the cobalt (ECP – before – 6.7 mg/l, after – 31.3 mg/l). The third patient's reaction to the acrylic plastic accompanied by increases in both the level of tryptase (before – 4.8 mg/l, after – 14.7 mg/l), and cationic protein (up to – 7.6 mg/l, after – 27.8 mg/l).

Conclusion:

- 1 A combination of determination of tryptase and ECP allows you to fix two different versions of immune system response to drug allergen.
- 2 Tryptase and ECP can be determined by standardised methods.
- 3 Detection of mediators in saliva before and after allergen provocation *in situ* can determine minimally invasive diagnostic rules for determining an allergic reaction.

476

Positivity criteria for the intradermal test in chlorhexidine allergy

Opstrup, MS^{1,2}; Mosbech, H²; Krøigaard, M²; Garvey, LH²

¹National Allergy Research Centre, Gentofte University Hospital, Hellerup, Denmark; ²Danish Anaesthesia Allergy Centre, Allergy Clinic, Gentofte University Hospital, Hellerup, Denmark

Background: Diagnosis of allergy to the widely used disinfectant chlorhexidine is based on several tests including skin prick

test (SPT), intradermal test (IDT), specific IgE and histamine release test (HR), since no standardised provocation model is available.

Interpretation of IDT is often difficult and several different positivity criteria exist. Therefore, the aim of this study was to evaluate three different criteria for positivity for IDT in patients diagnosed with allergy to chlorhexidine in the Danish Anaesthesia Allergy Centre (DAAC).

Method: In the period July 2004 – July 2012 a total of 343 patients were investigated in DAAC and allergy to chlorhexidine was diagnosed in 25 patients. Allergy to chlorhexidine was diagnosed if a minimum of two of the following tests were positive: SPT with chlorhexidine digluconate 5 mg/ml, specific IgE (Immunocap[®], Phadia AB, Sweden) and HR (RefLab ApS, Denmark). Criteria for positivity were: SPT wheal ≥ 3 mm of the negative control; specific IgE > 0.35 kUA/l; HR class ≥ 1 . Intradermal test with chlorhexidine digluconate 0.002 mg/ml was performed in duplicate in 333 patients. Three different criteria for evaluating the IDT were applied:

- (1) diameter of wheal \geq twice the negative control
- (2) diameter of wheal ≥ 3 mm larger than negative control and
- (3) diameter of wheal ≥ 8 mm (independent of size of negative control).

Results:

- (1) Diameter of wheal \geq twice the negative control was positive in 16/25 with allergy and 2/308 patients without allergy. Specificity 99.4%. Sensitivity 64%.
- (2) Diameter of wheal ≥ 3 mm larger than negative control was positive in 22/25 with allergy and 4/308 without allergy. Specificity 98.7%. Sensitivity 88%.
- (3) Diameter of wheal ≥ 8 mm was positive in 23/25 patients with allergy and 16/308 without allergy. Specificity 94.8%. Sensitivity 92%.

Conclusion: There is no international consensus on the criteria for a positive IDT and in this study the results differ greatly when applying different criteria. Our results show that when diagnosing chlorhexidine allergy in DAAC the highest combined specificity and sensitivity was achieved by using either diameter of wheal ≥ 3 mm larger than negative control or a wheal of a minimum of 8 mm. Unfortunately, a validated provocation model is not available for chlorhexidine allergy and future studies should investigate the positivity criteria for IDT for other drugs with a validated provocation model.

477

The diagnostic value of basophil activation tests in immediate radiocontrast media hypersensitivity

Demirel, F¹; Sener, O¹; Musabak, U¹; Gulec, M¹; Kartal, O¹; Yesillik, S¹; Baysan, A¹; Pekel, A¹; Sagkan, R¹

¹Department of Internal Medicine, Division of Immunology and Allergic Diseases, Gulhane Military Medical School, Ankara, Turkey

Introduction: In this study, we aimed to investigate the value of basophil activation tests (BAT) in the diagnosis of immediate radiocontrast media (RCM) hypersensitivity reactions and compare the results with the previous studies.

Method: Four groups (G) of patients were enrolled to the study. G1: seven patients with a history of immediate RCM reaction and with a positive RCM skin test; G2: nine patients with a history of early RCM reaction with a negative RCM skin test; G3: nine healthy volunteers who were used RCM without a history of RCM reaction; G4: 17 healthy volunteers who never were used RCM. CD63 and CD203c expressions were determined on basophils using flow cytometer. The percentages of active basophils, stimulation index (SI) and mean fluorescence intensity (MFI) values were used for comparisons after *in vitro* stimulation with 1/100 RCM dilution.

Results: The BAT results of G1 are $>5\%$ active basophil in four patients (57.1%), ≥ 2 SI in three patients (42.8%), increased MFI value in five patients (71.4%) and the BAT results of G2 are $>5\%$ active basophil in two patients (22.2%), ≥ 2 SI in one patient (11.1%), increased MFI value in one patient (11.1%), respectively.

Conclusion: Our findings suggest that BAT is not a useful tool based on the results of % active basophil, SI and MFI values. However, it may be considered as a promising technique when combined with RCM skin tests.

478

Sensitisation to proton pump inhibitors

Barasona Villarejo, MJ¹; García Nunez, I¹; De Castro Gómez, C¹; Moreno Aguilar, C¹; Guerra Pasadas, F¹

¹Hospital Reina Sofía, Allergology, Córdoba, Spain

Background: Increasing consumption of proton pump inhibitors (PPIs) in recent years (especially omeprazole) is increasing sensitisation to such drugs. We report a study to specify existing concrete sensitisation and in turn offer an alternative as a gastric protector.

Method: Four women with a mean age of 55 (44–69) showed reaction to different drugs, and a proton pump inhibitor was always involved. Only in one did the

reaction occur after taking lansoprazole alone. The other three women had a total of six episodes after taking different anti-inflammatories together with omeprazole. Of the seven episodes three presented dyspnoea. Medical assistance was needed in seven episodes.

The four patients underwent skin challenge test (SCT) and/or controlled exposure test with another different PPI to the one causing the reaction. A SCT was therefore carried out in two patients: one with pantoprazole and another with pantoprazole and omeprazole. Two patients were administered omeprazole, another two pantoprazole, one lansoprazole and another esomeprazole.

Results: In one patient the SCTs with omeprazole were negative, but the controlled challenge test (CCT) with omeprazole, the SCT with pantoprazole and the CCT with esomeprazole were positive. In another patient the CCT with omeprazole was positive but with pantoprazole was negative. In the third patient the SCT with lansoprazole was positive but she tolerated lansoprazole and the fourth patient was diagnosed with sensitisation to omeprazole and pantoprazole after positive CCT with the latter.

Conclusion: We report four cases of sensitisation to PPIs. Although the literature explains that reactions due to the use of these drugs are still infrequent, we must take them into account as a possible cause of reactions in order to recommend other proton pump inhibitors after carrying out an allergy study.

480

Clavulanic acid allergy

Rial Prado, MJ¹; Rico Díaz, A¹; Veleiro Perez, B¹; González Guzmán, LA¹; García Paz, V¹

¹Abente y Lago Hospital, Allergy, A Coruña, Spain

Background: Recently clavulanic acid allergen extract has been added to the Spanish market. The skin test with the extract can be performed for diagnosis of betalactams allergy.

Method: Skin tests with clavulanic acid were performed on 34 patients with a history of allergy due to amoxicillin or amoxicillin-clavulanic acid.

We performed prick test and intradermal at three concentrations 0.5 mg/ml, 5 mg/dl and 20 mg/ml with clavulanic acid and with PPL, MDM, Penicillin, Ampicillin, Amoxicillin and Cefuroxime. Patients with positive skin test to clavulanic acid and negative skin test to amoxicillin were challenged with Amoxicillin.

Results: From 23 patients with a history of allergy due to amoxicillin-clavulanic

acid, eight patients had a positive skin tests with clavulanic acid and negative result with amoxicillin skin tests.

Three patients had positive intradermal in all concentrations and five patients had positive skin tests in the concentration of 20 mg/ml exclusively.

Challenge with amoxicillin was performed in five of these patients with good tolerance. No adverse reactions were found with clavulanic acid skin tests.

Conclusion: Inclusion of clavulanic acid to study betalactams allergy should be considered as it is safe and can help rule out cases of allergy to amoxicillin.

However, further studies are needed to establish the sensitivity and specificity of this test.

481

Sensitisation to salicylates

Barasona Villarejo, MJ¹; Garcia Nunez, I¹; Perez Rodrigo, I²; De Castro Gómez, C¹; Moreno Aguilar, C¹; Guerra Pasadas, F¹

¹Hospital Reina Sofía, Allergology, Córdoba, Spain;

²Hospital Reina Sofía, Córdoba, Spain

Background: Although consumption of high-dose salicylates has decreased in recent years, there remain patients with sensitisation to these drugs. We demonstrate patients who are not NSAID intolerant but are allergic to aspirin.

Method: Fourteen patients were studied – all had come to the surgery due to reaction associated with the use of aspirin. All subjects underwent anamnesis with special focus on tolerance to anti-inflammatories tolerance and all patients underwent controlled challenge test (CCT) with ibuprofen 600 mg and diclofenac 50 mg.

Results: The 14 patients studied, 7 women and 7 men, mean age 43 years (20–71), jointly presented 20 episodes of reactions following the use of acetylsalicylic acid. Except in one episode this always occurred after the first dose and in 16 episodes treatment in the emergency department was needed to reverse the reaction. On no occasion was there any anaphylactic shock but in 12 episodes there were respiratory symptoms. In eight episodes the clinical diagnosis was labial, eyelid or facial angioedema and in four cases there was generalised urticaria. Twelve patients reported that they had subsequently tolerated anti-inflammatories without salicylates. All patients underwent controlled administration test with diclofenac 50 mg, and 12 of them who reported no known tolerance to ibuprofen underwent the CCT with the same drug. Both tests were carried out with a 1 week interval, with the results being negative in all cases.

Conclusion: It is important to consider the importance of a careful history in the study of allergy to anti-inflammatories, which will guide us to the existence or not of intolerance, although the definitive diagnosis of allergy to salicylates is given by the administration of ibuprofen and diclofenac.

482

The clinical pattern of hypersensitivity to non-steroidal antiinflammatory drugs and outcomes of pertinent oral challenges

Potika, M¹; Guibas, G¹; Koulias, C¹; Zeliou, CG¹; Chatzipetrou, A¹; Aggelides, X¹; Makris, M¹

¹Medical School, Athens University, University Hospital "Attikon", Drug Allergy Outpatient Clinic, Allergy Unit 'D. Kalogeromitros', 2nd Department of Dermatology and Venereology, Athens, Greece

Background: NSAIDs are one of the leading causes of adverse reactions to medications worldwide. Our aim is to present the clinical characteristics and the allergological evaluation of patients with suspected NSAIDs hypersensitivity.

Method: One hundred-twelve patients (♀ 84, mean age 48 years, range 19–84) who were referred to us during a 4-year period (2008–2012) with symptoms suggestive of NSAIDs hypersensitivity were evaluated retrospectively. A detailed medical history (indication, atopy, other drug allergy, culprit drug, time interval between drug intake and reaction, clinical manifestations) was recorded. Accordingly, single blind oral challenges (OC) to the offending or alternative agent and skin tests were performed when necessary. Upon conclusion, all patients were given written instructions for avoidance and NSAID therapeutic options.

Results: The usual culprit NSAIDs were: aspirin 28/111(25.2%), mefenamic acid 23/111(20.7%), paracetamol 17/111(15.2%) and diclofenac 14/111(12.5%). Time to reaction was <2 h in 75/112 patients and >2 h in 22/112; data was missing for 15pts. Systemic reactions were reported in 34/112pts (30.3%); 95.5% of the patients reported cutaneous manifestations, 24.3% respiratory and 8.9% cardiovascular symptoms. Concurrent diseases were: allergy to other drugs (48 cases), acute urticaria (33), asthma (13), chronic spontaneous urticaria (9 cases) and nasal polyps (5). Skin testing was positive in two cases involving paracetamol. Sixty-six out of 112 pts refused to undergo a provocation test or were lost in follow-up. Sixty one OC were carried out in 46 patients: 13/17 tolerated the suspect drug, whereas 4/17 -all provoked with paracetamol- had positive OC, solely with skin manifestations; OCs in aspirin or other

potent COX-1 inhibitor were also performed to evaluate cross-reactivity (2/17 positive to aspirin, 15/17 negative). Twenty-seven patients due to risk-benefit considerations or denial to consent, underwent OC with either the selective COX-2 inhibitor celecoxib (24/27) or the weak COX-1 inhibitor nimesulide (3/27), all of them uneventfully. Additionally, 5 aspirin sensitive patients underwent successful desensitisation due to coronary artery disease.

Conclusion: OC is the gold standard for the assessment of hypersensitivity reactions to NSAIDs. COX-2 or weak COX-1 inhibitors appear to be a safe alternative for NSAIDs sensitive patients with cutaneous manifestations.

483

Corticosteroids adverse reactions: our experience

Reyes, SM¹; Arruti, N¹; Muñoz, D¹; Audicana, TM¹; Longo, NM¹; Villarreal, O¹

¹Hospital Universitario Araba, Allergy, Vitoria, Spain

Background: Corticosteroids are widely used in the treatment of allergic disorders. The first reactions attributed to these drugs were described at the end of 1950s, since then several cases of allergic reactions have been published, being delayed type reactions the most widely studied. Cases of corticosteroid-induced adverse reaction referred to our consultation during the period 2005–2011 are reviewed in this study.

Method: A total of 189 patients were recruited, 77% (N 146) of them were women between 24 and 89 years old. In cases of drug- immediate reactions skin tests were carried out with a corticoids battery, with reading of results after 20 min. Wheals with 3 mm diameter were considered positive, as recommended by the European Academy of Allergy and Clinical Immunology. A basophil activation test (BAT) was also performed in five cases. In cases with history of delayed type reactions, skin tests with readings at 20 min and 24 h were performed. This group of patient underwent patch tests with a corticosteroid baseline series.

Controlled oral challenge were performed in the majority of the patients with an alternative drug in cases with positive results and with a drug involved in cases with negative results.

Results: In the patients studied, 19 cases showed a clinical history compatible with immediate reaction after administration of the corticosteroids, 14 out of them had a negative result, in five patients the results of the study were positive. Skin tests were positive in four cases. The BAT was posi-

tive in three cases and one of them was the only case showing a positive test. In 32 patients the clinical manifestation presented was allergic contact dermatitis (ACD), documented in 78% of cases by skin test and/or patch test. In the remaining cases the study was negative. Allergy to corticosteroids were discarded in 138 patients using a controlled oral challenge test with the involved drug. In 78 out of 138 cases the clinical reaction presented was only flushing after administration of corticoids, without involvement of an immune mechanism.

Conclusion: ACD was present in 25% of the cases, in this case a high diagnostic yield of the study was obtained (78%), being deflazacort, budesonide, betamethasone and dexamethasone, the most frequently involved drugs.

The best tolerated corticosteroid in all groups was methylprednisolone. The corticosteroid most frequently involved in all the groups was dexamethasone.

484

Hypersensitivity reactions to iodinated contrast media: clinical features and allergological work-up

Koulias, C¹; Potika, M¹; Chliva, C¹; Aggelides, X¹; Chatzipetrou, A¹; Makris, M¹

¹Medical School, Athens University, University Hospital 'Attikon', Drug Allergy Outpatient Clinic, Allergy Unit 'D. Kalogeromitros', 2nd Department of Dermatology and Venereology, Athens, Greece

Background: Iodinated contrast media (ICM) cause immediate and non-immediate hypersensitivity reactions, despite the introduction of nonionic, low osmolar ICM. Immunological mechanisms have been implicated recently in the pathogenesis of these reactions, as indicated by positive skin testing to ICM. Hence, skin testing has been integrated to the allergological work-up of patients presenting with ICM hypersensitivity, yielding best results when conducted within the time period of 2–6 months after the reaction.

Method: Patients referred to our Unit for ICM hypersensitivity reactions within the last 2 years underwent an allergological work-up consisting of: a/thorough medical history b/skin prick tests (SPTs) to undiluted and intradermal tests (IDTs) with 100-fold and 10-fold diluted solutions of the culprit ICM and at least two others -of different class- up to seven ICM, c/Patch tests (PTs) with the same battery of undiluted ICMs in cases of non-immediate reactions. The severity of immediate reactions was evaluated using the Ring and Messmer grading system.

Results: Twenty six patients (12♂/14♀) were evaluated: 10pts with immediate (4

grade 1, 4 grade 2, 1 grade 3 and 1 grade 4) and 16 with non-immediate reaction (maculopapular exanthemas in all cases). Atopy and drug allergies were reported by 7/26(27%) and 5/26(19%) pts respectively. Mean age at reaction was 58.2(22–75) years. The ICM was administered intravenously in 8/10 of immediate reactions and 3/16 of non-immediate. Iodixanol was the most common culprit drug (13/26), followed by iopamidol (3/26). The mean time to reaction after infusion was 10(1–30) minutes for immediate and 38(4–156) hours for non-immediate reactors.

Skin testing was negative in 25/26 cases. Seven patients were tested 2–6 months after the episode; the others (19/26) delayed mostly due to late referral (8–168 months, mean 45). One patient, with a grade 2 immediate reaction 2 months ago, was positive to all tested ICM (8 different agents).

Conclusion: In our well-selected population an extremely low percentage of positive tests were observed, although they were performed to various different ICM according to prior described methodology. The high time interval from reaction to skin testing could serve as a possible explanation for some cases, but not for all of them.

485

Drug reactions: results of 189 tests in patients from Policlínica Geral do Rio de Janeiro Allergic Clinic

Arcanjo, LC¹; Gonçalves, T¹; Rios, JL¹; Rios, J¹; Baroni, J¹

¹Policlínica Geral do Rio de Janeiro, Rio de Janeiro, Brazil

Background: In recent years there has been a better standardisation of allergy tests for adverse drug reactions allowing a better assessment of the patients who come to us with this complaint.

The objective is to investigate the frequency of positive suspected drugs tests, in patients with history of Drug Reactions.

Method: A retrospective study of medical records of patients treated at the Allergic Clinic of Policlínica Geral do Rio de Janeiro between September 2010 and September 2012. In this period, 189 drugs tests were performed.

Results: One hundred forty-nine of the 189 patients were female (78.8%). The majority of these women were between 41 and 60 years (48%), while in male the highest concentration occurred between 21 and 40 years (44% of males).

The drugs more frequently tested were: local anesthetics, 101 tests (54%); Antibiotics, 32 (17%), NSAIDs, 27 (14%);

iodinated contrast media, 17 (9%); other drugs 12 (6%).

Negative tests occurred in 179 (95%) patients, while 8 (4%, 6 female and 2 male) presented positive tests and 2 (1%) showed inconclusive results. Antibiotics were the more frequent positive tests: 3 for penicillin and one for amoxicillin, followed by 3 positive tests to NSAIDs and one for local anesthetics.

One hundred and thirty-six (72%) of the 189 patients tested, presented complaints strongly suggestive of adverse drug reaction (ADR). All the positive tests occurred among these patients and corresponded to 6% of them. No patient without a strongly suggestive ADR history a positive test.

Conclusion: This study reveals that in face a strongly suggestive history of adverse drug reaction, the respective tests should be performed, whereas in case of an unclear history the tests for drug allergy may be unnecessary.

486

Descriptive study of our betalactam allergic patients diagnosed in the first year after the last episode

García Nunez, I¹; Barasona Villarejo, MJ²; Algaba Marmol, MA²; Moreno Aguilar, C²; Guerra Pasadas, F²

¹Allergy Department, Hospital Quiron, Malaga, Spain;

²Allergy Department, Hospital Universitario Reina Sofía, Cordoba, Spain; ³Distrito Sanitario Osuna, Centro de Salud 'El Almorcón', Ecija, Spain

Background: Betalactam allergy suspicion is a very common problema referred by our patients. A rapid diagnose after the last episode could be cheaper and quickly than a study more than 1 year after the last episode. Our aim was to describe our patients with betalactam allergy suspicion in the first year after the episode and the protocol we performed.

Method: All patients coming to our Allergy Department from 2005 to 2009 with betalactam allergy and the last episode the year before the study were selected. A detailed anamnesis including specificity of involved penicillin, serum specific IgE (sIgE) levels and cutaneous tests to betalactams (CTBL) PPL, MDM, penicillin, ampicillin and amoxicillin-clavulanic acid in all cases, by means of skin prick test (SPT) and intradermal test (IDT). If a cephalosporin was involved, cutaneous test with cefuroxime, cephadroxile and ceftriaxone were done too. When negative CTBL response, we proposed to the patients a controlled oral administration (DPT) with amoxicillin-clavulanic acid.

Results: Forty-two patients (18 males and 24 females; mean age 42.61 years) were included in this study. Twenty-eight (66.66%) were diagnosed by specific IgE

levels, 9 (21.42%) by Cutaneous Tests and 5 (11.90%) by DPT. Thirty six patients (85.71%) referred an aminopenicillin as the implicated drug. No retest was needed to confirm the diagnose.

Conclusions:

- Specific IgE levels are very important to diagnose patients referring symptoms less than 1 year after the last episode.
- Although several patients need a DPT to confirm the diagnose, a rapid study is cheaper and more safety than a study several years after.
- Our protocol let us to diagnose quickly patients referring the last episode few months ago.

489

Heparin induced anaphylaxis in a dialysis dependent patient

Santosa, A¹; Tan, SH²; Cheng, YK^{2,3}

¹National University Health System, Singapore, Singapore; ²Gleneagles Medical Centre, Singapore, Singapore; ³Department of Medicine, Yong Loo Lin School of Medicine, Singapore, Singapore

Background: Heparin has been widely used for intra-dialytic anticoagulation since the 1940s. Heparin induced anaphylaxis can be life threatening, mandating early recognition and intervention. However, due to its relative rarity many physicians remain unaware.

Methods and Case Report: A 70-year-old Chinese female with hypertensive kidney disease was deemed to require dialysis. She had no known allergies and never received angiotensin converting enzyme (ACE) inhibitors. Dialysis was commenced through a permanent catheter with 500U of heparin/hour. She tolerated 3 hemodialysis sessions per-week in the initial 2 weeks. The following week she developed hypotension and dyspnea at the start of dialysis. First use syndrome (an anaphylactic reaction to the dialysis membrane) was the postulated cause and she was discharged after observation and intravenous hydrocortisone and antihistamines. However, she mounted a more severe reaction at start of the subsequent dialysis session 2 days later. A serum tryptase level by Fluorescent Enzyme Immunoassay (FEIA) done immediately after the reaction was elevated at 43.1 µg/l (ref < 11.4 µg/l). Heparin was thought to be the cause for her recurrent anaphylaxis. Further evaluation to confirm the suspected heparin allergy and determine safe alternatives was indicated.

Results: Skin prick tests were done with unfractionated heparin (5000 u/ml), dalteparin (2500 u/ml), enoxaparin (20 mg/

0.2 ml), tinzaparin (3500 IU/0.35 ml) and fraxiparin (2850 IU/0.35 ml). The prick test was positive for heparin but negative for the rest. This was further substantiated by a positive intradermal test with heparin at 1 : 10 concentration (negative at 1 : 1000 and 1 : 100). Intradermal tests were negative for dalteparin at all concentrations. This was followed by subcutaneous and intravenous challenges with dalteparin at incremental concentrations, reaching up to 250U of dalteparin. She has tolerated hemodialysis with dalteparin for the past 2 years.

Conclusions: The workup of a patient with intra-dialytic anaphylactic reactions must include a systematic evaluation. First use syndrome is rare with increased standards of sterilization and use of higher biocompatibility membranes. Similar reactions have also been reported to heparin contaminated with oversulfated chondroitin sulfate in 2008. Intra-dialytic anticoagulants must always be considered as a potential cause. Systematic workup to confirm the causal agent and to determine safe alternatives are crucial.

490

Allergy to paracetamol

Ortega, S¹; Morales, C¹; López, R²; Martorell, C¹; Raducan, I¹; Peláez, A¹

¹Hospital Clínico Universitario de Valencia, S. Alergia, Valencia, Spain; ²Hospital Universitario La Fe, S. Alergia, Valencia, Spain

Background: A 17-year-old woman with a history of allergic rhinoconjunctivitis resulting from sensitisation to dust mites and food allergy to peaches and cherries (LTP Syndrome). Recently the patient has sought medical care as she developed two episodes of urticaria (November, 2011 and February, 2012) two hours after food ingestion and approximately sixty minutes after taking 1 g of oral paracetamol. The patient has no recollection of the episodes coinciding with menstruation, exercise or other factors. Thereafter she has tolerated the same kind of food ingested on both occasions; she has not taken paracetamol in between or after the second food ingestion.

Method: The study was carried out 3 months after the last reaction. Intradermal tests with paracetamol (up to a concentration of 1 mg/ml) in the patient and in healthy controls.

Basophil activation test (BAT) (Basotest Glycotope Biotechnology) with paracetamol concentrations of 2 and 10 mg/ml. Stimulation index (SI) >2.0 was considered positive.

Oral provocation test with paracetamol and ibuprofen.

Skin prick tests and serum specific IgE related to the food ingested prior to the episodes as well as specific IgE to omega-5 gliadin (Tria a 19) (UniCAP ThermoScientific) were also performed.

Results: Skin tests and specific IgE to food: negative.

Skin tests with paracetamol in patient and controls: negative.

Oral provocation with paracetamol: positive (generalised urticaria, otic itching, throat inflammation and dyspnoea occurred ten minutes after a single 125 mg dose administration).

Repetition of intradermal tests with paracetamol 45 days after the provocation: positive (papule/erythema: 8/20 mm) to a concentration of 0.1 mg/ml.

BAT with paracetamol: positive (SI = 2.6).

Oral provocation with 600 mg ibuprofen: negative. Since February, 2012 the patient has tolerated NSAIDs on several occasions.

Conclusion: Allergic IgE reactions to paracetamol are not very frequent and often the mechanism can not be proved. In our case, the positive results of the skin tests as well as the BAT have allowed to confirm the IgE-mediated mechanism of the reaction.

It is a known fact that skin test sensitivity depends on the time elapsed after the reaction has occurred to carrying out the allergy study, which explains the negative results of the first skin test.

491

Insulin type III hypersensitivity reaction

Gomes, R¹; Loureiro, C¹; Calado, G¹; Faria, E¹; Segorbe Luis, A¹

¹Coimbra University Hospital Center, Coimbra, Portugal

Background: Insulin, a crucial therapeutic agent for diabetes mellitus (especially in type 1 patients) has been rarely associated with hypersensitivity events and the majority of the reported cases are IgE-mediated reactions.

Case Report: A 33 years old female patient, previously diagnosed with type 1 diabetes for about 8 years, was observed in an imunoallergology outpatient clinic in October 2012 for suspected insulin allergy. She was daily medicated with long acting insulin glargine (Lantus™) in the morning and intermediate acting insulin lispro (HumalogMix25™) according to the carbohydrates intake and glucose levels. In April 2012, for patient convenience, insulin therapy via an external infusion pump with insulin lispro was started. By the third month, she developed an exuberant reaction, at the infusion site, with nodulation,

hematoma and pain, without systemic or other associated symptoms. Perfusion was stopped, switching back to the initial regimen with LantusTM and HumalogMix 25TM. However, similar symptoms persisted (painful nodule and bruising) at the injection site 4 to 6 h after administration, resolving spontaneously in about 48 h. In addition, higher insulin doses were needed to achieve desirable glycemic control with the notion that the higher the dose the more severe the local reaction observed.

Methods and Results: We performed skin prick test to latex, skin prick-to-prick and

intradermal tests to regular human insulin (Humulin RegularTM), human insulin Act-rapidTM, an human insulin isophane suspension (HumulinNPHTM), insulin glargine (LantusTM) and a mixture of 75% insulin lispro protamine suspension and 25% insulin lispro (HumalogMix25TM), that were all negative. Specific IgE for human, bovine and porcine insulin, as also protamine, were negative. Suspecting of an immune complexes reaction type (type III hypersensitivity reaction), we decided to perform a skin biopsy (lesional and peri-lesional) that

revealed vasculitis with leukocytoclasia compatible with the suspected diagnostic.

Conclusion: With the evidence of an immune complexes-mediated reaction, desensitisation is not possible. There are no effective therapeutic attitudes described and the patient cannot avoid this treatment. Finally, the authors would like to emphasize the rarity of this situation, the likely misdiagnosis, the importance of a skin biopsy when a type III hypersensitivity reaction is suspected and therapeutic implications.

Poster Session 6

How to deal with complex drug allergies

492

Safety and efficacy of an adalimumab desensitisation protocol

Gutierrez Fernandez, D¹; Foncubierta Fernandez, A²; Anguita Carazo, JL³; Fernandez Melendez, S⁴; Fernandez Anguita, MJ⁵; Medina Varo, F⁶

¹Hospital Universitario Puerta del Mar, UGC Neumología-Alergia, Cadiz, Spain; ²Distrito Sanitario Bahía de Cádiz-La Janda, UGC Joaquín Pece, San Fernando, Spain; ³Complejo Hospitalario de Jaén, UGC Alergia, Jaén, Spain; ⁴Hospital Universitario Carlos Haya, UGC Alergia, Málaga, Spain; ⁵Hospital Universitario Puerta del Mar, UGC Farmacia, Cadiz, Spain; ⁶Hospital Universitario Puerta del Mar, UGC Reumatología, Cadiz, Spain

Background: We report the case of a 37-year-old female patient, diagnosed with rheumatoid arthritis refractory to conventional therapies, who initiated therapy with Adalimumab. Twelve days after the initial 40 mg subcutaneous dose, she experienced generalised urticaria and bilateral eyelid angioedema, attributable to a delayed reaction to the medication, requiring treatment with parenteral corticosteroids and H1-antihistamines to resolve the symptoms.

Method: Full allergy testing was carried out, including skin prick tests with Adalimumab, intradermal injections with 1 : 10 and 1 : 100 dilutions of the drug, patch tests, total and specific IgE measurements, and an 8-step desensitisation protocol was implemented, to evaluate the safety and efficacy of protocol.

Results: We recorded positive skin prick test responses to house dust mites, cat and dog dander, olive and grass pollen (and negative responses to hamster dander and latex). The prick tests with 50 mg/ml Adalimumab and with 1 : 10 and 1 : 100 dilutions of the drug, produced a delayed response, 5 days later, in the form of an erythematous papular lesion at the prick site. Total IgE level was 354 U/ml. Patch

tests with undiluted Adalimumab and with Adalimumab diluted in saline to 5% and 10% concentrations were applied to the original injection site in the upper back, with negative results. Premedication of 20 mg oral dexamethasone and an intramuscular dose of 5 mg dexchlorpheniramine were administered one hour before initiating the desensitisation protocol. The protocol consisted of a total of 8 doses of the drug, administered subcutaneously, which gradually increased to a cumulative dose of 40 mg (see Table 1). The protocol was well tolerated by the patient, with no immediate or delayed reactions to the various doses.

Conclusion: The application of an Adalimumab desensitisation protocol proved to be a safe and effective process which may be employed in those patients for whom there are few alternative therapies.

494

Progesterone induced urticaria and angioedema: induction of drug tolerance (desensitisation)

Kopyltsova, Y¹; Minikes, N¹; Kaplan, B¹

¹North Shore LIJ Medical Center, Allergy Immunology, Great Neck, NY, United States

Background: Progesterone induced urticaria and angioedema is a rare disorder, characterised by cyclic skin eruptions 10–14 days before the onset of menses. It is related to the spectrum of conditions termed autoimmune progesterone dermatitis. The manifestations range from recurrent erythema multiforme, papulovesicular rashes, to urticaria and angioedema. A clinical history of cyclic eruptions is supported by positive skin test to proges-

terone or a progesterone challenge. Treatment aims to suppress ovulation.

Method: We present a case of an 18-year-old girl. With onset of regular periods, she noticed intensely pruritic erythematous lower extremity rashes, occurring 14 days prior to her menses. The erythematous, pruritic, non-vesicular rash lasted 2–3 days. She also experienced nausea and vomiting for 3 days prior to menses. All symptoms resolved with onset of her period. A trial of multiple oral contraceptive pills (OCPs) did not improve her symptoms. Lupron was started to suppress her cycles. After the third cycle of Lupron, her cyclical symptoms disappeared. In May of 2012, she was given Lupron holiday. In the middle of her cycle she developed pruritic rash on the lower extremities, as well as difficulty swallowing and respiratory distress. Lupron was restarted. She suffered from hot flashes and significant weight gain over sixty pounds. She was referred for possible IDT to progesterone.

Results: Epicutaneous and intradermal skin test with progesterone (Depo-Provera) was positive at ID 1 : 10 dilution. The patient underwent a 3 day progesterone IOT, using Progesterone, immediate release formulation. She was able to tolerate target dose of 150 mg of Progesterone. Lupron was stopped.

Conclusion: We present a case of successful IOT to progesterone in a case of progesterone induced urticaria and angioedema in a patient, who failed other treatments. At 5 months follow up, patient continues to tolerate Progesterone without any systemic allergic symptoms. Occasionally she still gets mild rashes.

495

Successful induction of drug tolerance (desensitisation) to lopinavir/ritonavir in a patient with multidrug-resistant AIDS and drug hypersensitivity

Diaz, JM¹; Kopyltsova, Y¹; Kaplan, B¹

¹Allergy and Immunology, North Shore-LIJ Health System, Great Neck, NY, United States

Background: Long-term antiviral therapy is a standard of care for advanced HIV disease. Reportedly, patients with HIV have about a 100-fold greater risk of devel-

Table 1 Adalimumab desensitization protocol

Dose	Dose (mg)	Cumulative Dose (mg)	Blood Pressure (mmHg)	Arterial Pulse	Adverse Reactions
1	0.003	0.003	136/89	110	None
2	0.030	0.033	110/71	95	None
3	0.300	0.333	121/77	90	None
4	1.000	1.333	122/86	93	None
5	2.000	3.333	115/78	86	None
6	5.000	8.333	123/79	89	None
7	10.000	18.333	130/92	99	None
8	12.000	40.333	116/78	86	None

oping a hypersensitivity drug reaction. In this case we present a successful induction of drug tolerance to lopinavir/ritonavir in a multidrug-resistant patient with AIDS and drug hypersensitivity.

Method: Case report.

Results: Our patient is a 41 year-old female with AIDS (CD4 count: 27 and viral load >27 000) who failed multiple antiretroviral regimens due to viral resistance and hypersensitivity reactions. In 2011, 8 days after starting lopinavir/ritonavir she developed diffuse hives. Since then, she has attempted to restart lopinavir/ritonavir four times with similar reaction occurring eight to twelve days into treatment. There were no associated respiratory symptoms or angioedema. Due to low CD4 count, high viral load and extensive ART resistance, it was imperative to her care to start lopinavir/ritonavir. As part of IDT our patient received eight doses of lopinavir/ritonavir (80 mg/20 mg/ml suspension) at twenty minute intervals on day one and her goal dose on day two. The goal dose for our patient was 400 mg lopinavir/100 mg ritonavir. Our dosing increments were based on lopinavir dose: 4, 8, 16, 24, 40, 64, 100 and 144 mg on day one. She was monitored for an hour after final dose without any adverse events. On day two she was able to tolerate the full dose of lopinavir/ritonavir (400/100 mg). Four months after her IDT, she continues to tolerate lopinavir/ritonavir with improvement in her CD4 count as well as a dramatic drop in her viral load.

Conclusion: To our knowledge this is the first case report of IDT to lopinavir/ritonavir in an AIDS patient. Through this case we are able to provide a successful protocol for lopinavir/ritonavir induction of tolerance in a patient with delayed onset of urticaria.

496

Successful desensitisation to mecasermine in an allergic patient with primary IGF-I deficiency

Gutierrez Fernandez, D¹; Lopez Martinez, I²; Lechuga Sancho, AM²; Foncubierta Fernández, A³; Anguita Fernandez, MJ²; Lechuga Campoy, JL⁴

¹Hospital Universitario Puerta del Mar, UGC Neumología-Alergia, Cádiz, Spain; ²Hospital Universitario Puerta del Mar, UGC Pediatría, Cádiz, Spain; ³Distrito Sanitario Bahía de Cádiz-La Janda, UGC Joaquín Pece, San Fernando, Spain; ⁴Departamento Materno Infantil y Radiología, Facultad de Medicina de la Universidad de Cádiz, Cádiz, Spain

Background: Allergic reactions to mecasermine have been reported in approximately 8% of patients receiving Mecasermine, for various indications in several clinical trials. However, only twice have such reactions been published in patients with severe pri-

Table 1 Mecasermine desensitization protocol

Dose	Dose (mg)	Cumulative Dose (mg)	Adverse Reactions
1	0.01	0.01	None
2	0.02	0.03	None
3	0.04	0.07	None
4	0.08	0.15	None
5	0.16	0.31	None
6	0.32	0.63	None
7	0.64	1.27	None
8	1.15	2.42	None

mary IGF-I deficit, which is currently the only approved indication. We report a patient with severe primary IGF-I deficit and a history of respiratory and food allergies who developed raised itchy wheals on legs, arms and thorax on the tenth day of treatment.

Method: Full allergy testing was carried out, including skin prick test with 10 mg/ml mecasermine, intradermal injections with 1 : 10 and 1 : 100 dilutions of the drug, total and specific IgE measurements. We performed a desensitisation regimen on eight steps with increasing concentrations of mecasermine at 15 min intervals, up to the 10 mg/ml concentration of the commercial preparation. The protocol was implemented at 0.12 mg/kg which is the maximum required by the patient for growth optimisation. Premedication with deflazacort, dexchlorpheniramine and montelukast was employed one hour prior to implementation of the protocol.

Results: The skin prick test for pneumoallergens were positive to house dust mite. Total IgE was upper 100 kU/l and specific IgE tests were positive to house dust mite and egg white. The prick test with 10 mg/ml mecasermine and intradermal injections with 1 : 10 and 1 : 100 dilutions of the drug were negative. The 8-step desensitisation protocol, shown in Table 1, was completed in two hours. The patient has now been under therapy for 4 weeks, with a notorious growth response, and no further allergic reactions of any kind.

Conclusion: We present the first successful desensitisation protocol to mecasermine, to our knowledge, in a patient with severe IGF-I deficiency and an atopic background.

497

Rapid response of severe acute generalised exanthematous pustulosis to infliximab

Yawalkar, N¹; Lee, HY¹; Heidemeyer, K¹

¹Department of Dermatology, University of Bern, Bern, Switzerland

Background: Acute generalised exanthematous pustulosis (AGEP) is a rare cutaneous adverse drug reaction characterised by an

acute onset of widespread non-follicular sterile pustules arising on erythematous basis in association with fever and peripheral leukocytosis. AGEP has been linked to intake of various drugs, most commonly antibiotics. Clinical course of this reaction is mostly benign and the majority of cases resolve within 15 days after discontinuation of the responsible medication.

Method: Report of a case. Immunohistochemical analysis of TNF- α in a skin lesion prior to therapy with a TNF-antagonist.

Results: We here describe a cases of AGEP with development of multiple concomitant targetoid lesions resembling a Stevens-Johnson syndrome-like reaction induced by terbinafine. Since progression of the exanthema despite cessation of the causing drug and systemic corticosteroid therapy was observed, therapy with infliximab (5 mg/kg bodyweight), a TNF-antagonist, was initiated. Rapid resolution of disease with clearance of pustular lesions was noted within 3 days. Immunohistochemical analysis of a skin lesion obtained prior to therapy with infliximab demonstrated strong expression of TNF- α .

Conclusion: This case demonstrates that TNF- α plays a central role in the pathogenesis of severe forms of acute generalised exanthematous pustulosis and that TNF-antagonists can be helpful in recalcitrant cases.

498

Successful desensitisation to rituximab in four patients with autoimmune diseases

Ramírez, LF¹; Cañas, CA²; Tobon, GJ²; Bonilla, F²; Serrano, CD¹

¹Fundación Valle del Lili, Allergy Unit, Cali, Colombia; ²Fundación Valle del Lili, Rheumatology Unit, Cali, Colombia

Background: Rituximab is a chimeric monoclonal anti CD20 antibody used frequently in the treatment of some hematologic malignancies and autoimmune diseases. Infusion-related reactions are common and no standardised strategy is approved to the treatment of them, apart of premedication. Underlying mechanism of reactions include massive cytokine release, hypersensitivity, immune or cytokine imbalance, cross reactivity, and symptoms not directly affecting the immune system. In the case of rituximab, cytokine release seems to be the most common. Our aims were to describe a new short desensitisation protocol to rituximab and to establish if IgE is involved in these reactions.

Methods: Patients with autoimmune diseases in which rituximab had induced hypersensitivity reactions were included. A

six hours intravenous desensitisation protocol was used. Premedication with hydrocortisone, loratadine and acetaminophen were given to all patients one hour before the onset of the infusions. Prick and intradermal test were made at concentration of 1 mg/ml, previous to the first desensitisation in each patient.

Results: Four patients (three women and one man) were included. Two of them had systemic lupus erythematosus, one reumatoid arthritis, and one reumatoid arthritis and Sjogren syndrome. Previous reactions included skin manifestations (urticaria and/or angioedema) in all of them and dyspnea in one. None of the reactions were presented within the first cycle of rituximab and all occurred between few minutes and three hours of the onset of the infusion. Prick test and IDR were negative in all. A total of seven desensitisations were carried out and all patients tolerated them. Minor side effects were present in four of the seven protocols (three patients). None of the infusions had to be stopped at any time.

Conclusion: In these patients, infusion-related reactions to rituximab seem to be not related to IgE sensitisation. However, a short desensitisation protocol was successful in them. Large scale studies are needed to recommend the use of desensitisation in patients with non allergic hypersensitivity to rituximab.

499

Multiple drug allergy due to polyethylene glycols hypersensitivity

Badiu, I¹; Nebiolo, F¹; Pizzimenti, S¹; Bussolino, C¹; Raie, A¹; Rolla, G¹

¹Mauriziano Hospital, University of Torino, Torino, Italy

Background: Polyethylene glycols (macrogols or PEG) are condensation products of glycols with ethylene oxide, leading to molecules of various length: polysorbate 80, macrogol 400, 4000, 6000 ecc. Macrogols are widely used as excipients in foods, cosmetics, and topical and systemic drugs because of their stabilising properties. Immediate hypersensitivity reactions have been reported after oral and parenteral administration of products containing macrogols, such as tablets, vaccines, and laxative oral solutions. We report here the first case, to our knowledge, of a hypersensitivity reaction due to different polyethylene glycols products.

Case Report: A 26-year-old woman, with no previous history of allergy, reported many episodes of generalised urticaria one hour after the intake of antibiotics (amoxicillin clavulanate and ciprofloxacin tablets)

and antiinflammatory drugs (ketoprofen granules and diclofenac tablets).

Diagnostic work-up: specific IgE antibody determinations (benzylpenicillin, ampicillin, and amoxicillin), and skin tests with major determinants (penicilloyl-polylysine) and minor determinants (minor determinant mixture) of benzylpenicillin and amoxicillin-clavulanic acid resulted negative. Oral challenge with amoxicillin clavulanate caused diffuse urticaria 30 min after assumption of 55 mg of drug. Prick test 1/1000 for ciprofloxacin 10 mg/ml resulted positive (wheal diam. 10 mm). Oral challenge with alternative antiinflammatory drugs (meloxicam, imidazole salicylate and etoricoxib) caused diffuse urticaria shortly after taking small doses of these drugs. We noticed that all the test drugs (including cutaneous ciprofloxacin) contained PEG with different length (from 80 to 6000). Prick test resulted positive for all polyethylene glycol derivatives: polysorbate 80 (prick test 1/1000, wheal diam. 20 mm), PEG 400 and 4000 (prick test 1/1000, wheal diam. 10 mm), PEG 6000 (prick test 1/100, wheal diam. 20 mm). Basophil activation tests for polysorbate 80, PEG 400, 4000 and 6000 were all negative. Specific IgE for ethylene oxide was negative. Oral challenge with etoricoxib, amoxicillin clavulanate and ciprofloxacin tablets with no PEG were all well-tolerated.

Conclusion: Polyethylene glycols derivatives have been described responsible of non-immunological reactions similar to those of our patient. The case we report here is the first case of multiple drugs reaction due to different polyethylene glycols hypersensitivity, with mechanisms which remain elusive.

500

Anaphylactoid reaction during haemodialysis: a case report

Piñero-Saavedra, M¹; Prados Castaño, M¹; Gonzalez-Quevedo Tejerina, T¹; Leguisamo, S¹; Quiralte, J¹

¹Virgen del Rocío University Hospital, Allergy Unit, Sevilla, Spain

Background: Low-molecular weight heparins (LMWH) are important anticoagulants. They are used in the prophylaxis and treatment of thromboembolic disorders. Allergic reactions to LMWH are rare. The most common reactions involve erythematous plaques and maculopapular exanthema. We present an anaphylactoid reaction due to enoxaparin.

Method: A 51 year-old man with a history of chronic kidney disease stage V presented, in the 5th session of his first cycle of dialysis, after 5 min of the beginning of the process: an intensive flushing, tachycardia, bronchospasm, tightness, tachypnea

and dyspnea which required treatment with corticosteroids and oxygen. Enoxaparin was the only administered drug prior to dialysis. In the following session we changed the protocol and administered the drug only at the end of haemodialysis session. The patient stayed asymptomatic all along the session and symptoms only appeared again 5 min after enoxaparin injection. Our study included: tryptase levels on acute episode, blood count cell and skin prick test with enoxaparin, bermiparin, nadroparin, dalteparin and fondaparinux. We added basophil activation test (BAT) with enoxaparin and fondaparinux, and after informed consent, we proceed to controlled oral challenge with fondaparinux.

Results: Laboratory test were normal, including platelet count and tryptase levels. Skin prick test and BAT were all negative. The controlled oral challenge with fondaparinux has been well-tolerated.

Conclusion: Differently to inespecific and delayed hypersensitivity reactions, anaphylactoid reactions to LMWH are very infrequent. In anaphylactoid reactions to LMWH, when allergic study is negative, unfractionated heparins could be a safe option.

501

Desensitisation to clopidogrel: a tailor-made protocol

Barreira, P¹; Cadinha, S¹; Malheiro, D¹; Moreira da Silva, JP¹

¹Centro Hospitalar de Vila Nova de Gaia/Espinho, EPE, Drug Allergy Unit, Allergy and Clinical Immunology Department, Vila Nova de Gaia, Portugal

Background: Clopidogrel is an antiplatelet drug widely used for treatment and prevention of a variety of cardiovascular diseases. Its combination with acetylsalicylic acid (ASA) is considered essential to reduce the risk of stent thrombosis.

Method: We report a successful desensitisation to clopidogrel in a patient with delayed hypersensitivity (HS).

Results: A 70-year-old Caucasian man with allergic rhinitis, hypertension and dyslipidemia was referred to our outpatient clinic for suspected drug allergy. Long-standing medication included ramipril, carvedilol, nitrates, indapamide and ASA. He was submitted to coronary catheterization with placement of a stent and prescribed clopidogrel 75 mg/d. After 2 weeks, he developed lip, hand and foot swelling, erythematous papular non pruritic lesions and arthralgias that were treated with corticosteroids and antihistamines. Cutaneous lesions resolved in 5 days and arthralgias in 3 weeks. Laboratory evaluation revealed no abnormalities. Patch tests

with different concentrations of clopidogrel (10%, 30% and 50% pet) and oral challenge were negative. One week later, the patient started continuous administration of the drug and 24 h after a single dose developed erythematous papular pruritic lesions. Skin biopsy suggested drug related dermatitis. He was advised to avoid clopidogrel and treatment with ticlopidine was started but it was not tolerated (gastrointestinal symptoms). Since dual antiplatelet therapy was required and therapeutic alternatives to clopidogrel are limited, a 3 h desensitisation protocol was started, achieving a cumulative dose of 154 mg without any reaction and a daily dose of 75 mg was recommended. On the 4th day, the patient developed skin lesions similar to the previously described. He was treated with topical steroids and oral antihistamines and the daily dose of clopidogrel was reduced to 20 mg. A new desensitisation protocol was established, with a slow dose increment, according to the patient's response. It was only possible to achieve the dose of 75 mg/d after 2 months. One month later, although clopidogrel was well tolerated, the patient decided to stop its administration.

Conclusion: Although well tolerated by most patients, HS reactions with clopidogrel may occur. Since other treatment options are limited and frequently not tolerated, desensitisation is rising as a safe alternative in those patients. In delayed reactions with cutaneous lesions, a slower desensitisation protocol may be necessary.

502

Allergic reaction to chlorpheniramine maleate: a case report

Demirel, F¹; Gulec, M¹; Kartal, O¹; Yesillik, S¹; Baysan, A¹; Musabak, U¹; Sener, O¹

¹Department of Internal Medicine, Division of Immunology and Allergic Diseases, Gulhane Military Medical School, Ankara, Turkey

Introduction: Despite the frequent use of antihistamines in daily practice, allergic reactions to these drugs are extremely rare. Herein, a systemic reaction against chlorpheniramine maleate (CM) used for allergy prophylaxis was reported.

Case report: A thirty-nine-year-old female patient was given CM for a suspected radiocontrast media allergy before coronary angiography. The symptoms and signs of systemic reactions occurred within minutes after the intravenous administration. Four weeks later, skin tests and basophil activation tests (BAT) with CM was performed to evaluate the allergic status. The stimulation index was 1.29 in BAT. Prick test result was found negative with different

dilutions of CM. However, following intradermal test with 1/100 W/Vol dilution of CM both local and systemic symptoms occurred in seconds. The patient was treated with adrenalin and steroid injections intramuscularly.

Discussion and Conclusion: Although she had never used antihistamines for any reason previously, CM sensitisation can be explained by a possible cross-reaction with some other drugs such as flu medications. It should be kept in mind that widely used antihistamines treating allergic diseases may cause allergic reactions.

Keywords: Chlorpheniramine maleate, basophil activation test

503

Immediate type hypersensitivity reaction to levothyroxine and oral desensitisation

Demirel, F¹; Gulec, M¹; Kartal, O¹; Tas, C²; Baysan, A¹; Yesillik, S¹; Musabak, U¹; Sener, O¹

¹Department of Internal Medicine, Division of Immunology and Allergic Diseases, Gulhane Military Medical School, Ankara, Turkey; ²Department of Pharmaceutical Sciences, Gulhane Military Medical School, Ankara, Turkey

Introduction: Hypersensitivity reactions against levothyroxine are very rare. In this report, a levothyroxine hypersensitivity reaction and subsequent successful desensitisation process is presented.

Case report: A 35-year-old female patient, who has already followed up by our clinic because of multiple drug allergy, applied with the symptoms of a systemic reaction occurred within half an hour after the intake of levothyroxine tablets. A stock solution was prepared by dissolving 25 mcg levothyroxine tablet in 1 ml volume of 0.09% phosphate buffered saline according to a previously published protocol to evaluate levothyroxine allergy. Prick test performed with undiluted stock solution and intradermal test with 1/100 W/vol were negative. Subsequently, intradermal test with 1/10 W/vol concentration was found positive and a desensitisation was planned. Since any oral levothyroxine desensitisation protocol was not found in the literature, the desensitisation scheme was planned according to the general desensitisation rules. This process lasted 2 days and she was able to take 75 µg levothyroxine orally on the third day without any problem. Levothyroxine therapy has been continued successfully since then.

Conclusion: As far as we know, no oral desensitisation protocol was defined for levothyroxine in the literature. Current desensitisation method can be used as an example for levothyroxine allergy treatment.

Keywords: Levothyroxine allergy, desensitisation

504

Procarbazine hypersensitivity and tolerance induction

Pascolini, L¹; Nucera, E¹; Buonomo, A¹; Pecora, V¹; Rizzi, A¹; Aruanno, A¹; Ricci, G¹; Di Rienzo, A¹; Mezzacappa, S¹; Centrone, M¹; Colagiovanni, A¹; Schiavino, D¹

¹Università Cattolica del Sacro Cuore, Rome, Italy

Background: Procarbazine is an alkylating agent used for treatment of brain tumors. It is often well tolerated, however, 6–8% of treated patients developed macular-papular rashes or urticaria. Most of reactions caused by procarbazine are non immune-mediated. In the literature there are no reports about tolerance induction to procarbazine. We report a case of tolerance induction in a pediatric patient with a clinical history of hypersensitivity reaction to procarbazine.

Method: This is a case of 6 years old child affected by glioma. He was in therapy with procarbazine (100 mg) and dexamethasone (0.6 mg) daily. During the first cycle of chemotherapy he developed generalised urticaria and so the treatment was stopped. He started the second cycle but he presented generalised urticaria again so patient was hospitalised.

Result: Patient referred to our Allergy Department and he underwent to allergological examination. Procarbazine is only available in capsule form so skin prick test (SPT) were performed using undiluted commercial preparation (50 mg/ml) prepared by dissolving the powder in saline. Histamine was used as a positive control. Patch tests were performed using the same drug as for SPT according to ENDA recommendations. SPT and patch tests resulted negative and we concluded for a nonallergic drug hypersensitivity reaction. Previous parental authority, the patient was underwent to a protocol of oral desensitisation to procarbazine. Gradually increasing doses of procarbazine were administered in 5 days. Each dose was administered every 20 min. On the first day, the patient received 0.15 mg of procarbazine and the final dose of 100 mg was achieved. Before the initiation of the protocol were administered cetirizine 10 mg/ml (20 drops) every day. Patient received the first desensitisation in our department and continued to intake procarbazine at home at the dose of 100 mg/day for 12 days without adverse reactions.

Conclusion: In our case there is no chemotherapeutic drug as an alternative to procarbazine so the desensitisation was necessary. Most of rechallenger with procarbazine in

patients with adverse reactions were positive and the drug be stopped. Desensitisation is a high risk treatment and it is mainly indicated in IgE-mediated reactions but it is effective even in non IgE-mediated hypersensitivity reactions.

505

Successful desensitisation with propylthiouracil in two patients

Demirel, F¹; Gulec, M¹; Kartal, O¹; Yesilik, S¹; Baysan, A¹; Musabak, U¹; Tas, C²; Aydogdu, A³; Sener, O¹

¹Department of Internal Medicine, Division of Immunology and Allergic Diseases, Gulhane Military Medical School, Ankara, Turkey; ²Department of Pharmaceutical Sciences, Gulhane Military Medical School, Ankara, Turkey; ³Department of Internal Medicine, Division of Endocrinology and Metabolic Diseases, Gulhane Military Medical School, Ankara, Turkey

Introduction: Despite the frequent use of antithyroid drugs, propylthiouracil and methimazole, allergic reactions to these drugs are very rare. Herein, a systemic reaction to propylthiouracil and subsequent successful oral desensitisation was presented in two patients.

Case report: A 41-year-old female and a 27-year-old male patients were referred to our clinic with the histories of systemic allergic reactions to both methimazole and propylthiouracil. The symptoms and signs of active hyperthyroidism were determined at the physical examination. The skin tests with propylthiouracil and methimazole could not have been done because of ongoing antihistamine therapy. The patients' symptoms related to antihyperthyroid drugs were thought to be caused by a possible type 1 hypersensitivity reaction. With the importance of the continuation of antihyperthyroid therapy, a desensitisation process with propylthiouracil was performed. Since any propylthiouracil desensitisation protocol was not found in the literature, the desensitisation scheme was planned according to the general desensitisation rules. This process lasted 1 day for female patient and 2 days for male patient and both of the patients were able to take 300 mg propylthiouracil orally at the end of the desensitisation without any problem. The propylthiouracil therapy has been continued successfully since then.

Conclusion: As far as we know, any desensitisation protocol was not defined for propylthiouracil in the literature. This desensitisation method can be used as an example for propylthiouracil allergy treatment.

506

A pediatric protocol for monoclonal antibodies rapid desensitisation

Caimmi, D^{1,2}; Caimmi, S¹; De Amici, M¹; Bosa, L¹; Demoly, P²; Marseglia, GL¹

¹Pediatrics, University of Pavia- IRCCS Policlinico San Matteo, Pavia, Italy; ²Pneumology and Addictology, Hôpital Arnaud de Villeneuve, Université de Montpellier, Montpellier, France

Background: Monoclonal antibodies are important therapeutic tools, but their usefulness may be limited when patients experience acute hypersensitivity reactions. Patients who have a history of reaction risk to be ruled out of their therapeutic protocol or have to switch to an alternative drug. Drug desensitisation has proven to be a highly effective strategy to re-administer a drug in patients who experienced a hypersensitivity reaction. It consists in forcing the patient's immune system to accept and tolerate the allergen, but such a procedure is still empiric for monoclonal antibodies and not common in the pediatric population. The aim of our study was to demonstrate the clinical success of a new protocol of rapid desensitisation to infliximab in a pediatric patient, affected by ulcerative colitis (UC).

Method: A 14 years old patient affected by a severe form of UC experienced an anaphylactic reaction, involving the skin and the respiratory apparatus, at the third dose of infliximab. The administered dose was 5 mg of infliximab per kg (250 mg total dose). Infliximab is a chimeric immunoglobulin G1k monoclonal antibody that binds with high affinity and specificity both to the soluble form and to the membrane-bound TNF- α . Infliximab is used both for induction and maintenance of remission in Crohn's disease, and in the treatment of UC. After the first reaction, the patient underwent skin tests to infliximab (skin prick test at 10 mg/ml, and intradermal test at 1 mg/ml), which resulted negative, while a provocation test to the drug resulted positive 6 min after administration of the first dose (0.1% of the total dose: 2.5 mg). The reaction included dyspnea, bronchospasm, and angioedema of the face. Tryptase levels resulted normal, while histamine doubled compared to basal levels; basophil activation test was negative. We used a 13-steps protocol in which the final cumulative dose was 250 mg, and the first was 1/1 000 000 of the total dose. We have tripled the dose at each step, every 15 min.

Results: Rapid desensitisation was not interrupted for the appearance of any reaction and the patient tolerated the drug. He has already received twice his treatment since we introduced this new protocol.

Conclusion: We hypothesise the patient experienced a non-IgE mediated hypersen-

sitivity reaction to infliximab, but the protocol we used has been useful to administer him the appropriate treatment.

507

Drug induced recurrent angioedema in a patient with active pulmonary tuberculosis, chronic obstructive pulmonary disease and history of chronic urticaria

Leru, PM¹; Bozdog, OB²

¹Allergology, Colentina Clinical Hospital, Bucharest, Romania; ²Dermatology, Elias Emergency Hospital, Bucharest, Romania

Background: Acquired angioedema is an allergic disease with increasing prevalence in clinical practice and significant difficulties regarding triggers and treatment. Comorbidities and complex medication represent important risk factors for allergic diseases in adult atopic patients.

Case: We present the case of a 48 years old man, 30 pack-units smoker, with a history of mild chronic urticaria, who came for recurrent episodes of facial angioedema and cutaneous pruritus. He was diagnosed with active cavitary pulmonary tuberculosis 1 month before and received daily antituberculosis therapy with four drugs, associated with allopurinol for moderate hyperuricemia, omeprazole and oral B6 vitamin. Chronic obstructive pulmonary disease (COPD) stage II GOLD was diagnosed based on chronic cough and nonreversible obstruction on spirometry. Allergologic evaluation showed very high plasma level of total IgE (2300 UI/ml), with normal specific IgE to current food and inhalant allergens. Immunologic tests, complement fractions and serology for viral, bacterial and parasitic infections were normal. We performed basophil degranulation test for all drugs used, which gave significant high values for allopurinol and moderate values for ethambutol. The lymphocyte blast transformation tests to drugs were negative. We administered combined nonsedating antihistaminic therapy in maximum doses in order to avoid corticotherapy. The episodes of angioedema continued about 2 months, with progressive reduced intensity and duration, even after discontinuation of allopurinol and ethambutol. The clinical, bacteriologic and immunologic evolution after 6 month was good, with sputum negativation and resolution of pulmonary lesions. The total IgE remained in high levels, probably due to multifactorial etiology. The skin tests will be performed after the end of therapy and stop of antihistamines, according to the patient decision.

Conclusion: Allopurinol is a well known trigger for drug induced allergic diseases, sometimes severe in association with other chronic medication. We consider that multidrug therapy, associated with chronic diseases and active infectious are important triggers for recurrent angioedema in adults.

anemias associated with chronic kidney disease. Allergic reactions to erythropoietins and darbepoetins have only occasionally been reported. These skin reactions include pruritus, wheals at the injection site, orofacial anaphylaxis and anjioedema. In this article, we report an 11 year-old female who experienced generalised erithematous

skin eruption and desquamation after both erythropoietin and darbepoetin treatments. We successfully used darbepoetin with the support of premedication and desensitisation. Because of the erythropoietin or darbepoetin treatment was so necessary in these allergic patients; our approach might be a suitable option for them.

508

Darbepoetin desensitisation: a case report

Tahan, F¹; Akar, HH¹; Dursun, I²; Yilmaz, K²

¹Department of Pediatric Allergy, Erciyes University School of Medicine, Kayseri, Turkey; ²Department of Pediatric Nephrology, Erciyes University School of Medicine, Kayseri, Turkey

Human recombinant erythropoietins (EPO) and darbepoetins are widely used for

Poster Session 7

Drug allergy: anaphylaxis

509

Anaphylaxis in a tertiary adult allergy clinic: a retrospective review of 516 patients

Gelincik, A¹; Demirturk, M¹; Yilmaz, E¹; Ertek, B¹; Unal, D¹; Colakoglu, B¹; Buyukozturk, S¹

¹Department of Internal Medicine, Division of Allergy, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey

Background: Anaphylaxis is a life-threatening acute allergic reaction that can occur at any age. Our aim was to determine the frequency, triggering factors and clinical features of anaphylaxis among adult patients who were referred to a tertiary health care facility.

Method: A retrospective medical chart review was performed including all patients referred to the outpatient clinic of the adult allergy department in our university hospital between January 1, 2008 and December 30, 2011 in order to determine cases involving anaphylaxis.

Results: A total of 516 (2.11%) patients among 24 443 admissions were diagnosed with anaphylaxis. Although the second highest frequency of anaphylaxis cases took place in 2008, a gradual rise in the frequency was determined from 2009 to 2011. Drugs (90.7%) were the most frequent cause, followed by Hymenoptera stings (5.4%), foods (1.6%), latex (0.4%) and exercise (0.2%) respectively. The clinical manifestations during anaphylaxis reported by patients were cutaneous (n:292, 56.6%), respiratory (n: 253, 49%), cardiovascular (n: 212, 41%), neuro-psychiatric (n:60, 11.6%) and gastrointestinal (n: 52, 10.1%), respectively. Approximately one-fifth of the patients received epinephrine whereas 43% of patients did not receive epinephrine during their treatment in the emergency room. An epinephrine auto-injector was prescribed to 42 patients (8.1%).

Conclusion: In this study, the second pattern of NIAID/FAAN diagnostic criteria for anaphylaxis predominated among adult patients. Drugs were the leading triggering factor followed by Hymenoptera stings, foods, latex and exercise, respectively. Atopy, asthma and allergic rhinitis were rarely detected.

510

Anaphylaxis and drug allergy: one year evaluation

Gonzalez Ruiz, AM¹; Moreno Montoya, A¹; Moreno Rodilla, E¹; Ponce Guevara, LV²; Gracia Bara, MT²; Laffond Iges, E¹; Davila González, I¹

¹Alergología, Hospital Universitario de Salamanca, Salamanca, Spain; ²Hospital Universitario de Salamanca, Salamanca, Spain

Background: Anaphylactic reactions cause the maximal grade of allergic reactions and are potentially life-threatening. Drugs are the most common agents involved in anaphylactic reactions of adults and betalactam antibiotics seem to be the main cause.

Method: We studied 568 patients older than fourteen years with suspected drug allergy, seen in the allergy unit of the University Hospital of Salamanca (Spain), throughout the year 2012. The aim of this study was to describe the drugs involved and methods used for diagnosing patients who had suffered an episode of anaphylaxis related to drugs.

Results: A total of 37 Patients (6.5%) suffered one episode of anaphylaxis. The principal drugs involved in the anaphylactic reactions were betalactam antibiotics (62%), fluoroquinolones (9.5%), non-steroidal antiinflammatory drug (NSAIDs) (9.5%), iodinated contrasts (6%), and other drugs (13%) such as local anaesthetic, sulfonamides and neuromuscular blockers (rocuronium).

After the allergy work-up 16 (43%) patients had positive skin tests (prick or intradermal), one of those also had positive specific IgE by ImmunoCAP[®], 4 (11%) had a positive specific IgE with negative skin tests. Two (6%) of the patients with negative skin test and negative specific IgE had a positive drug challenge. In 9 (24%) patients the drug challenge was not performed due to the medical history or contraindications to the drug challenge.

In five patients the final diagnosis was idiopathic anaphylaxis after rule out drug allergy by drug challenge with the suspected drug. One patient was diagnosed of exercise-induced anaphylaxis.

Conclusion: In our series betalactam antibiotics were the main drugs involved in the anaphylaxis. We observed that the sensitivity of the skin tests was high in betalactam allergy but low in the other cases.

The most common drug involved was amoxicillin and not penicillin, this result is in line with the tendency observed since the 1980s. Fluoroquinolones and NSAIDs were the following drugs implied and these results support different studies indicating an increasing tendency of allergic reactions of these drugs.

512

Three cases of lansoprazole anaphylaxis with cross-reactivity to omeprazole: take home lessons

Koca Kalkan, İ¹; Kalpaklıoğlu, AF¹

¹Department of Immunologic and Allergic Diseases, Kirikkale University Faculty of Medicine, Kirikkale, Turkey

Background: Proton pump inhibitors (PPIs) are widely used for the treatment of acid-related gastrointestinal diseases, because they reduce the gastric acid secretion by blocking the H¹/K¹-ATPase. PPIs are generally well tolerated, and side effects occur in about 1% of the patients. There have been several reports of hypersensitivity reactions, but anaphylaxis is very rare. We present three patients with lansoprazole induced anaphylactic reactions that were diagnosed by skin tests and oral provocation tests (OPT).

Method: The first two patients referred to our allergy department with a history of adverse reactions to NSAIDs. First patient had experienced an episode of urticaria, angioedema in 30 min, while the second patient had experienced pruritic generalised skin rash with hypotension within two hours of drug intake. But the OPT with the culprit NSAIDs turned out to be negative. By detailing their clinical history, we found out that they were using lansoprazole along with NSAIDs. Third patient visited emergency room complaining of pruritus on her arms, dyspnea, hypotension and syncope in 30 min after taking lansoprazole. Following written informed consents, patients were challenged with lansoprazole, and with other PPIs; omeprazole, esomeprazole, pantoprazole and rabeprazole, in order to offer a safe alternative. Skin prick tests and intradermal tests were done. If negative, single-blind OPT were performed.

Results: In first two cases intradermal tests with omeprazole were positive and they developed anaphylaxis after lansoprazole during oral provocation test. Third patient showed positive result only with lansoprazole at skin prick and intradermal test.

Conclusion: Diagnosis of PPI allergy is difficult, since they are frequently used in combination with antibiotics or NSAIDs, and even without medical prescription. Therefore, a high index of suspicion should always be kept in mind. We present three patients with lansoprazole induced anaphylactic reactions, whose clinical features of the reactions suggest an IgE-mediated mechanism, as demonstrated by a positive skin test. Although previously lansoprazole was offered as a valid alternative PPI for omeprazole allergy, two of our patients had positive skin tests to omeprazole. So, we believe that there is no safe alternative for PPI allergy and skin/oral challenge tests should be done before offering a safe alternative to the patients.

513

Severe anaphylactic reaction after skin prick tests to betalactam antibiotics

Zande, M¹; Sinaniotis, A¹; Xenochristou, P¹; Panta, V¹; Psarros, P²; Syrigos, K³; Syrigou, E¹

¹Allergy, General Hospital 'Sotiria', Athens, Greece;

²Allergy, Athens Naval Hospital, Athens, Greece;

³Medical School, University of Athens, Athens, Greece

Background: Skin testing is the diagnostic cornerstone for IgE-mediated allergies and is considered extremely safe. It is usually performed with the prick and the prick-to-prick method. The aim of this study is to report a case of systemic anaphylactic reaction during skin prick tests (SPT) with b-lactamic antibiotics and to underline that anaphylaxis during SPTs is fortunately a rare but existing possibility.

Method: A 43 years old male patient came to our allergy outpatient clinic for drug allergy evaluation approximately 6 months after the initial incidence. According to his medical history five minutes after the first dose of amoxicillin in the reported episode, he presented generalised redness, stomach ache, tachycardia, dyspnea, difficulty in swallowing, dizziness and loss of consciousness. It is mentioned that he had taken amoxicillin 10 years ago. Initially *in vitro* tests were done for the detection of specific IgE CAP/FEIA c1, c2, c5, c6, c7 and k82 which were all negative, total IgE:30.2 KU/l. Patient medical history: hypertension. Medication: olmesartan. He was skin prick tested to various b-lactamic antibiotics (all together PPL1:1, MDM 1:1,

amoxicillin:20 mg/ml, ampicillin:20 mg/ml, cefuroxime:2 mg/ml, negative and positive control).

Results: During the 5 min of the test he complained for oropharyngeal pruritus, abdominal pain and he developed generalised urticaria and conjunctivitis. The skin tests to amoxicillin and ampicillin were strongly positive (wheal 25 × 19, and 15 × 12 respectively) and positive to MDM. The symptoms were treated with administration of adrenaline (IM), corticosteroids and antihistamines (IV). The ECG conducted was normal and he was examined by an ENT doctor without any abnormal finding. Blood samples were sent for tryptase: 17.7 µg/l. The baseline measurement was 5.2 µg/l. Since the patient has fully recovered, he was discharged with instructions of avoiding b lactam antibiotics and a complete emergency set for anaphylaxis with written instructions.

Conclusion: Anaphylaxis after SPTs for b lactams is a rare but possible, especially in high risk patients, phenomenon. As a result allergists should be always alert in order to diagnose and treat properly this life threatening condition.

514

Biphasic anaphylaxis to gemifloxacin

Yilmaz, I¹; Dogan, S²; Tutar, N¹; Kanbay, A¹;

Buyukoglan, H¹; Demir, R¹

¹Department of Chest Diseases, Erciyes University School of Medicine, Kayseri, Turkey; ²Department of Gastroenterology, Erciyes University School of Medicine, Kayseri, Turkey

Background: We report here in the case of a 60-year-old male who was taking on ACE inhibitor, α and β blockers and experienced a severe, resistant and biphasic anaphylactic reaction to gemifloxacin mesylate.

Case history: Five minutes after the first dose of gemifloxacin mesylate, he developed numbness around his mouth, itching particularly localised in the palmar and plantar regions, followed by fainting, clammy sweating, shivering, shortness of breath, dyspnea, facial and hand swelling. He was immediately admitted to the emergency department. On admission: his blood pressure was measured as 60/40 mmHg, and his pulse rate was 65 beats/min. Epinephrine was repeated three times with a five minute interval (0.3, 0.3, 0.5, respectively). Intravenous vasopressor treatment (Dopamine) was started due to the patient's blood pressure not increasing despite intravenous fluid and intramuscular epinephrine treatment. The patient was then promptly transferred to the intensive care unit in our hospital. On physical examination: Pulse rate of 60 beats/min,

blood pressure of 90/60 mmHg. He was taking on angiotensin-converting enzyme (ACE) inhibitor, Carvedilol and Tamsulosin hydrochloride. Atropine (IM) and ipratropium bromide monohydrate by nebuliser were added. Patient's symptoms were relieved; his blood pressure, pulse rate returned to normal within a few hours. He developed hypotension (blood pressure decreased to 50/30 mmHg) and itching-erythema five hours after the first reaction. Epinephrine 0.1 mg/ml (IV) and atropine 0.5 mg (IM) were injected. Methylprednisolone 80 mg (IV), ranitidine 50 mg (IV), and diphenhydramine 25 mg (IM) were administered. Meanwhile, glucagon was prepared. However, glucagon was not injected because the episode was resolved by the intravenous administration of aqueous epinephrine. One hour later, all of his symptoms had resolved and dopamine was stopped because of his experiencing high blood pressure in the following period.

Conclusion: If shock is imminent or has already developed, epinephrine needs to be given by slow intravenous route. A severe, resistant, biphasic anaphylactic reaction must be queried in cases with an ACE inhibitor, alpha and beta blocker drug history. Glucagon should be kept ready for patients taking an α and/or β-adrenergic blocker who have hypotension and bradycardia and who do not respond optimally to epinephrine and atropine.

515

Anaphylaxis to atracurium: unusual gastrointestinal features, data on timing of allergy investigation and acute tryptase sampling

Dua, S¹; Brennan, LJ¹; Jani, P¹; Ewan, PW¹

¹Allergy Department, Addenbrooke's Hospital, Cambridge, United Kingdom

Background: Anaphylaxis during general anaesthesia (GA) is a rare but life threatening event. During anaesthesia, anaphylaxis most commonly presents with cardiovascular collapse or respiratory difficulty. To our knowledge, gastrointestinal involvement has not been reported in the literature. Investigation of anaphylaxis requires the use of acute serum tryptase measurement and skin tests to determine aetiology. The timing of these tests is important

Method: A 64 year old man was admitted for laser excision of a vocal cord tumour under GA. Induction was with midazolam, propofol, remifentanyl and atracurium. He immediately developed profound hypotension, erythema and a fall in oxygen saturations. Anaphylaxis was diagnosed and treated with IV hydrocortisone, chlorpheniramine, fluids and metaraminol.

Cardiovascular collapse was easily reversed and although adrenaline was drawn up it was not given as it was not required. Surgery was abandoned. Thirty minutes after the onset of reaction, in recovery, he developed profuse diarrhoea and stomach cramps which continued for a further 40 min.

Results: Acute tryptase was 194, 155, 22 and 11.4 ng/ml at 30 min, 1, 12 and 24 h respectively. A clinic baseline taken 1 week after the event was 6.8 ng/ml. Skin tests were performed at 24 h post reaction and were weakly positive to atracurium. Due to the proximity to treatment skin tests were repeated a week later and were again positive to atracurium and in addition to cisatracurium and mivacurium (not administered). Anaphylaxis to atracurium was confirmed.

Conclusion: This case provides new data on several issues. To our knowledge gastrointestinal involvement has never been reported in the context of anaphylaxis due to neuromuscular blocking agents. Furthermore, in some allergy guidelines for the investigation of GA anaphylaxis, a delay of 6 weeks is suggested before allergy testing can be undertaken, without supporting data. This is the first data to show that allergy testing can be undertaken early after surgery and yield positive results. Lastly data on the time course of tryptase in acute reactions is limited with scarce data on GA reactions. This case provides time course data showing peak levels were higher at 30 min than one hour, supporting sampling immediately after resuscitation to ensure peak levels are not missed.

516

Allergy to short-acting b2-agonists in a patient with chronic obstructive pulmonary disease

Valbuena, T¹; Manso, L¹; Padial, MA¹; Reche, M¹; Pascual, CY¹

¹University Hospital Infanta Sofia, San Sebastian de los Reyes, Madrid, Spain

Background: Short-acting B2-agonists are the first line drugs for treating reversible airway obstruction, such as in asthma and in certain patients with chronic obstructive pulmonary disease (COPD). 'Paradoxical' bronchoconstriction following inhalation of B2-agonists has been documented.

Methods and Results: A 60-year-old man diagnosed with COPD, suffered a near-fatal respiratory exacerbation during the month of May. He was sent to our outpatient clinic to study the possible implication of pollen allergy. At the time of his visit in June, he was asymptomatic and treated with salmeterol/fluticasone. We performed skin prick tests (SPTs) for common

allergens (positive for *Olea europea*), spirometry (measurement of forced expiratory volume in 1 s [FEV1] and forced vital capacity [FVC]) and measurement of the improvement of lung function after inhalation of bronchodilator. The spirometry values were normal: 3280 (89%), FEV1 4180 (84%), FEV1/FVC 78%, and the bronchodilator test was negative. We saw the patient the following year and he did not have any symptoms during the pollen season and was controlled of his COPD. We repeated the measurement of lung function and five minutes after 200 µg of inhaled salbutamol, the patient experienced generalised itching and erythema, chest tightness with audible wheezes and a 46% drop in FEV1 (from 2.45 to 1.52 l). SPT with salbutamol, terbutaline and latex were negative and intradermal test with terbutaline was also negative. As the patient had an anaphylactic reaction to salbutamol and he needed a short-acting B2 agonist we decided to perform a challenge with terbutaline. The patient signed an informed consent statement for drug challenge and was then administered one inhalation of terbutaline and five minutes after he felt breathless, had cough and audible wheezes and a 35% drop in VEF1 (to 1.78 l). A basophile activation test (BAT) to salbutamol and terbutaline was performed next, but no activation was detected. As the patient had had two positive challenges to short-acting B2 -agonists we decided to perform challenges with long-acting B2-agonists (salmeterol and formoterol) which were well tolerated.

Conclusion: We present a case of an anaphylactic reaction after salbutamol administration and bronchoconstriction after terbutaline administration in which the immunological mechanism involved is unclear.

517

Anaphylaxis due to barley contained in a chocolate

Uriarte, SA¹; Bartolomé, B²; Ruiz, M¹; Cuesta, J¹; de las Heras, M¹

¹Allergy Department, Fundación Jiménez Díaz, Madrid, Spain; ²Bial Aristegui, I+D, Bilbao, Spain

Background: Chocolates and confectionary products contain nuts, cereals, additives and other ingredients capable to elicit allergic reactions. The aim of the study is describe a case of allergy due to barley contained in a chocolate and identify the responsible allergens.

Method: A 32-year old-man, with pollinosis and allergy to fruits (peach and banana), suffered oral pruritus, facial angioedema, generalised urticaria, dysphagia and dizziness

immediately after the ingestion of Malteser[®] chocolates, which contain milk, cocoa, malt, barley, wheat, soy and pectin, and are nut free. He had previously allergic reaction to beer. Skin tests, specific IgE determination and oral challenge were performed. Allergens were studied by SDS-PAGE, IgE-immunoblotting and inhibition assays.

Results: Prick tests were positive to grass pollens, cat epithelium, alternaria, peach, banana, pear, nuts, cereals (barley, oat and corn) and peach LTP. Prick-prick was strongly positive to barley flour and seed, beer and to the malt biscuit inside the Malteser chocolate. Skin tests were negative to cocoa, milk, pectin, soy, wheat, rye, rice, enzymes, profilin and other fruits. Oral challenge tests were negative to all raw and roasted nuts. Specific IgE by CAP and microarray ISAC was positive to lolium, Alt a 1, Fel d 1, Pru p 3, Jug r 3, barley, malt, oat, corn, banana and nuts. EAST IgE was also positive to barley seed and to Malteser biscuit extract. SDS-PAGE and Immunoblotting under non-denaturing condition revealed an extense IgE binding protein to barley seed and Malteser biscuit extracts, mainly at 17 kDa and high mw protein bands. IgE reactivity was also observed at 13–14 kDa common bands to banana and walnut extracts. IgE-immunoblotting under reducing condition showed a single 10 kDa and a prominent 14.5 kDa band in the barley seed and Malteser biscuit extracts, respectively. Barley seed extract caused a complete inhibition of specific IgE-reactivity to the 14.5 kDa Malteser biscuit extract, indicating the presence of cross-reactive allergens. No inhibition to barley seed and Malteser biscuit IgE-immunoblotting could be achieved with Pru p 3 patient serum pre-incubation.

Conclusion: We present a patient allergic to beer, peach and banana, with anaphylaxis to barley contained as an unexpected allergen in a confectionary product. The 10 kDa responsible allergen could correspond to barley LTP, despite no cross reactivity could be demonstrated between barley and Pru p 3.

518

Acute urticaria following folic acid injection

Zirbs, M^{1,2}; Seifert, F¹; Radopoulou, E^{1,2}; Cifuentes, L^{1,2}; Pfab, F^{1,2}; Ring, J^{1,2}; Darsow, U^{1,2,3}

¹Dermatology, Klinik und Poliklinik für Dermatologie und Allergologie am Biederstein Technische Universität München, Munich, Germany; ²Christine Kühne Center for Allergy Research and Education (CK-CARE), Technische Universität München, Munich, Germany;

³Center of Allergy and Environment, Technische Universität und Helmholtz Center, Munich, Germany

Background: Folic acid (pteroylmonoglutamic acid) is the synthetic form of folate

B vitamins, which naturally can be found in leafy vegetables, liver, yeast and milk. It is often added to multivitamin tablets and other groceries. In case of deficiency, pure folic acid is administered. Allergies to folic acid have been occasionally published and in one case could be verified by oral provocation.

Method and Results: We present a 81-year-old male patient, who suffered of an acute urticaria and dyspnea after injection of different vitamins including folic acid.

Allergologic laboratory assessment revealed a sensitisation against D. pteronyssinus and cat epithelia, as well as an elevated total serum IgE of 333 IU/ml. In the skin prick test a positive reaction was seen with folic acid, with a strong wheal (5 mm diameter) and flare (10 mm diameter) reaction. The other vitamins tested remained negative.

Patch testing with the accused substances was negative.

Double-blind, placebo-controlled titrated oral challenge confirmed hypersensitivity against folic acid (flush, pruritus, blurred vision and dizziness). The other accused vitamins and the placebo control were well tolerated.

Conclusion: Natural sources of folic acid in groceries (pteroylpolyglutamate and pteroylmonoglutamate) didn't have any impact on the patient. These substances are reduced in the intestine to tetrahydrofolic acid and probably have no allergenic potential in our patient. Accordingly, an allergy passport to synthetic folic acid was issued.

We recommended to the patient to pay attention to a sufficient supply of natural folic acid in his daily food intake.

palliative chemotherapy regimens. She suffered several episodes of systemic infusion reactions by Taxanes and Carboplatin. After a new Cisplatin protocol in the 4th cycle she developed an immediate severe reaction characterised by flushing, generalised pruritus, dizziness, dyspnea, diarrhea, abdominal cramping, hypotension (85/45 mmHg) and required emergency treatment and hospitalisation in the Intensive Care Unit. A tryptase level of 54.5 µg/l was observed after about 6 h of the reaction. Baseline levels were normal.

Method and Results: Skin tests:

Skin prick test (SPT) with common inhalants and food allergens: negative.

Carboplatin SPT (10 mg/ml) positive.

Cisplatin SPT (1 mg/ml) positive.

Oxaliplatin SPT (5 mg/ml) negative.

Intradermal test (0.5 and 5 mg/ml) negative.

Basophil activation test: Carboplatin positive (activation 85%), Cisplatin positive (activation 75%), Oxaliplatin negative. Controls negative.

A modified, previously reported, desensitisation protocol was performed with increasing Carboplatin doses at different concentrations (Solution A 0.0028 mg/ml in 50 ml; Solution B 0.028 mg/ml in 50 ml; Solution C 0.288 mg/ml in 100 ml; Solution D 1.44 mg/ml in 500 ml) in continuous infusion. A total cumulative dose of 720 mg was achieved in 7 h. Our patient has received successfully 3 carboplatin cycles with just mild reactions in the 2nd and 3rd cycle.

Conclusion: We have demonstrated the effectiveness of reported desensitisation protocols in our high-risk patient with a IgE mediated anaphylactic shock to platinum drugs.

nary tract infection and started with anal itching. She applied hemorrhoid creams in anal region and took dexchlorpheniramine. 7–8 h later she developed generalised urticaria and pruritus. The creams were discontinued. Immediately after the next dose of dexchlorpheniramine she developed generalised urticaria, pruritus, dizzy, decay, diarrhea and breathlessness. The medication was discontinued and the symptoms resolved completely in several hours without treatment. Two months later she took bilastine 20 mg and started 2 h later with the same symptoms, not needing treatment for this episode either. Four months later took diphenhydramine to sleep and several hours later began with generalised urticaria improved without treatment.

Results: Skin patch-tests with standard true-test and antihemorrhoidal cream were performed with negative results after 48 and 96 h reading. Skin prick-tests with desloratadine, cetirizine, loratadine, levocetirizine, ebastine, dexchlorpheniramine, and hydroxyzine (crushed and diluted in saline) were negative. Oral and ocular challenge tests with the same products, placebo, fosfomycin-trometamol, levocabastine and azelastine eye drops were negative except for dexchlorpheniramine, bilastine and ebastine, with a positive result. Basophil activation test (BAT) was positive for diphenhydramine and ebastine.

Conclusion: We present a case of anaphylaxis with several antihistaminics that was confirmed by oral challenge test. Although the mechanism of hypersensitivity to antihistamines is unknown, BAT positivity suggests an IgE-mediated mechanism in our case.

To our knowledge this is the first case of bilastine hypersensitivity.

519

Successful carboplatin desensitisation

Maghfour Martín, Y¹; Porcel Carreño, S¹; Gómez Nieves, E¹; Ahmida, T¹; Cámara Hijón, C²; Hernández Arbeiza, J¹

¹Allergology Department, Virgen de la Montaña Hospital, Cáceres, Spain; ²Immunology and Molecular Genetic Department, San Pedro de Alcántara Hospital, Cáceres, Spain

Background: Severe allergic reactions by chemotherapy drugs in oncologic patients represent a problem when there are no alternative treatment so desensitisation is necessary in many cases.

Case Report: A 41 year old woman was referred to our department due to a severe anaphylactic shock reaction by cisplatin. She had a previous history of adverse reaction to radio-contrast media without any other atopic conditions. She was diagnosed of poor outcome stage IB2 high level cervix carcinoma and treated with multiple

520

Antihistamines anaphylaxis including bilastine

Zambonino Carreiras, MA¹; Bobadilla Gonzalez, P¹; Garcia Menaya, JM¹; Jimenez Ferrera, G¹; Corrales Vargas, SI¹; Cordobes Duran, C²

¹Allergy Service, Infanta Cristina Hospital, Badajoz, Spain; ²Allergy Service, Merida Hospital, Merida, Spain

Background: H1-antihistamines are the most used drugs in allergic diseases. Bilastine is a new H1-antagonist used for the symptomatic treatment of allergic rhinoconjunctivitis and urticaria in adults or children over 12 years old.

We present a case of anaphylaxis after taking several antihistamines.

Method: A 44-year-old woman with a history of hemorrhoids (surgically treated 6 years ago) and hypothyroidism was treated with fosfomycin-trometamol for uri-

521

Ornipressin for the treatment of adrenaline-resistant anaphylaxis due to latex

Seedat, RY¹; van der Westhuizen, J²

¹Otorhinolaryngology, University of the Free State, Bloemfontein, South Africa; ²Bloemfontein, Private Practice, South Africa

Background: Anaphylaxis is defined as 'a serious allergic reaction that is rapid in onset and might cause death'. Adrenaline is the first-line treatment for anaphylaxis.

Method: We report the case of a patient with intraoperative anaphylaxis who was treated with intravenous ornipressin after a failure to respond to adrenaline.

Results: A 36 year old female developed anaphylaxis intraoperatively while undergoing an arthroscopy under general anaesthesia. She was known to have

hypothyroidism Eltroxin and known to have allergic rhinitis with sensitisation to cat and dog hair but was not asthmatic.

During induction, she had received ketamine, propofol and sufentanil and received ketotifen, dexamethasone and clindamycin ten minutes later. Two minutes after this, she developed flushing, bronchospasm, bradycardia and hypotension. She was treated with 0.5 mg of adrenaline intravenously every 2 min, 6 mg of etilefrine and 1 l of Ringer's lactate. Despite receiving 2 mg of adrenaline intravenously, she remained hypotensive with a bradycardia. She was also given 200 mg of hydrocortisone, 25 mg of promethazine and 50 mg of ranitidine intravenously. Following the administration of 1 IU of ornipressin, the bradycardia and hypotension resolved.

ImmunoCAP RAST testing performed after discharge from hospital was positive with a specific IgE of 5.06 IU/ml.

Ornipressin is a synthetic vasopressin analogue with potent and specific constrictor effect on the microcirculation and veins through its actions on the V1 receptor which is equal to, if not greater than, that of vasopressin. It is used locally to induce ischaemia and haemostasis at operative sites during ENT, gynaecologic and urologic procedures. Infusion of ornipressin causes a rise in arterial blood pressure by increasing the venous tone and decreasing the capacity of the peripheral circulation thus increasing peripheral vascular resis-

tance and shunting blood back to the vital organs and the central circulation.

Conclusion: Although adrenaline-resistant anaphylaxis is rare, clinicians need to be aware of the entity and the role of ornipressin in its management.

522

Two cases developing allergic reactions with both meloxicam and nimesulide

Keren, M¹; Köycü, G¹; Öner Erkeköl, F¹

¹Atatürk Chest Disease and Thoracic Surgery Training and Research Hospital, Immunology and Allergy Department, Ankara, Turkey

Background: The patients who have non-steroidal antiinflammatory drug (NSAID) intolerance should be tested for safe analgesic. In our country only the preferential COX2 inhibitors (meloxicam and nimesulide) are available. However, rare systemic reactions to these COX-2 inhibitors have been presented. Two cases who had allergic reactions with both of the drugs during tests for safe alternative analgesic will be presented. Their management plan will be discussed.

Case Presentation: Both cases had asthma and one of them had additional nasal polyposis. Both patients gave multiple allergic reaction history with different NSAIDs. The first case had respiratory and urticaria type intolerance reactions.

The second case described only respiratory system symptoms with consumption of NSAIDs. The first case developed runny nose and sneezing after oral provocation test (OPT) with meloxicam in the first hour. Twenty days after that reaction, we performed OPT with nimesulide. Five minutes after the first dose (a quarter of the usual dose) nausea, vomiting, abdominal pain and hypotension appeared. In the second case; 5.5 h after the OPT with nimesulide, the patient had shortness of breath. Rhonchi in physical examination and a 25% fall in FEV1 were detected. One week later we performed OPT with meloxicam. Four hours after the test, we observed flushing, hives, difficulty in swallowing and a 20% fall in FEV1. Afterwards, taking into account the patients' need for analgesics, aspirin desensitisation performed. The desensitisation processes were successfully completed in both cases. After desensitisation the patient tolerated NSAIDs including nimesulide and meloxicam.

Conclusion: Meloxicam and nimesulide preferentially inhibit COX-2, however high enough concentrations can inhibit the COX-1 enzyme. The COX-1 inhibitor activity of the drugs is the mechanism that explains the cases. ASA desensitisation can be an alternative treatment strategy for patients who can not tolerate any NSAIDs.

Poster Session 8

Understanding the mechanism of asthmatic inflammation

523

Collagen receptors: $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins are involved in eosinophil transmigration through human microvascular endothelial cell lung monolayer

Zuk, J¹; Bazan-Socha, S¹; Marcinkiewicz, C²; Zareba, L³; Plutecka, H¹; Mlicka, E¹; Musial, J¹
¹Jagiellonian University School of Medicine, Krakow, Poland; ²Temple University, Philadelphia, PA, United States; ³University of Rzeszow, Rzeszow, Poland

Background: Recruitment of the inflammatory cells to the airways is mediated by adhesive molecules. Among integrins, the most important in cell trafficking are those containing α_4 and β_2 subunits. We hypothesised that in bronchial asthma also collagen integrin receptors: $\alpha_1\beta_1$ and $\alpha_2\beta_1$, may be involved in cell migration to the inflammatory site. We recently described increased expression of both: α_1 and α_2 subunits on blood eosinophils and α_2 on CD4 T lymphocytes in asthma. The aim of the study was to analyse effect of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrin inhibition on transmigration of eosinophils and peripheral blood mononuclear cells (PBMC) through human microvascular endothelial cell lung monolayer in 12 atopic asthmatics and 12 healthy controls. We analysed also CD4/CD8 ratio in PBMC population before and after transmigration assay.

Methods: PBMC were separated by gradient centrifugation; eosinophils by gradient centrifugation and negative magnetic separation. For inhibition purposes we used snake venom derived anti-adhesive proteins: viperistatin, VP12, VLO5 and VLO4 (potent and selective inhibitors of $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins, respectively).

Results: In both groups of subjects all anti-adhesive proteins inhibited eosinophil, but only VLO5 and VLO4 PBMC transmigration; CD8 T cells migrated better than CD4 in control samples, but their transmigration was decreased after incubation with anti-adhesive proteins.

Conclusion: Both studied collagen receptors: $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins are likely to be involved in eosinophil transmigration to the inflammatory site of asthmatic subjects. The role of $\alpha_2\beta_1$ on lymphocyte is probably different. As a stimulator of collagen accumulation this integrin could be, at

least in part, responsible for asthma airway remodelling.

524

In vitro MUC5AC production and the effect of antioxidant in asthma angiotensin converting enzyme

Shim, J-J¹; Hur, G-Y¹; Min, K-H¹; Lee, S-Y¹; Kang, K-H¹
¹Korea University College of Medicine, Seoul, Korea

Background: Both bronchial asthma and chronic obstructive pulmonary disease (COPD) are characterised by chronic persistent inflammatory process, but the nature of the inflammation is different markedly. However, in real practice, both may occur concurrently in some patients, and such conditions have been defined as overlap syndrome. Therefore, we purposed to evaluate the mucus production in asthma, COPD and overlap syndrome in airway epithelial cell line, and to analyze the effect of antioxidants in mucus production.

Method: Cigarette smoke extracts (CSE) were withdrawn into a polypropylene syringe (50 ml) at a rate of one puff/min (5 times) and then bubbled slowly. We used NCI-H292 cell lines, stimulated by IL-13 (10 ng/ml), TNF- α (20 ng/ml) and cigarette smoking extracts (CSE). MUC5AC production was measured by immunoassay. Epigallocatechin-3-gallate (EGCG; 10, 20, and 50 μ M; Sigma Co. St. Louis, MO, USA) was pretreated 1 h before adding the cigarette smoke extract or cytokines.

Results: Co-treatment of IL-13 and TNF- α increased MUC5AC protein production in NCI-H292 cells ($P < 0.05$). CSE treatment also increased MUC5AC protein level in cells ($P < 0.05$). Treatment with IL-13, TNF- α and CSE more increased MUC5AC level than single treatment of them. Treated with EGCG (10, 20, and 50 μ M) decreased MUC5AC level dose dependently ($P < 0.05$, compared to untreated).

Conclusion: Mucus production in airway epithelial cells is increased in both asthma and COPD model. In overlap syndrome between asthma and COPD, mucus production is more increased compared to single asthma and COPD. Antioxidant treatment may helpful to reduce airway mucus secretion in overlap syndrome.

525

Significant correlation between the intensity of a positive skin prick tests and the prevalence of asthma in patients with pollinosis in Spain

Subiza, J¹; Fernández-Caldas, E²; Pola, J³; Moral, Á⁴; Feo, F⁵

¹Subiza Asthma and Allergy Centre, Madrid, Spain; ²Inmunotek Lab, Madrid, Spain; ³Sagasta Polyclinic, Zaragoza, Spain; ⁴Virgen Del Valle Hospital, Toledo, Spain; ⁵General Hospital, Allergy Unit, Ciudad Real, Spain

Background: Some, but not all, pollinosis patients with allergic rhinitis suffer from asthma. One of the more plausible explanations is the systemic propagation of the inflammatory mediators from the nose to the lower airways (Braunstaal GJ. JACI 2001, 107:469–476). Therefore, patients with severe allergic rhinitis would be more likely to suffer asthma. The aim of this study was to investigate if there is a significant correlation between the intensity of positive skin prick tests and the prevalence of asthma in pollinosis patients.

Material and Methods: Thirteen allergy clinics from 13 Spanish cities participated in this study. Patients were selected on the basis of a history of seasonal, or perennial allergic rhinitis and/or asthma. From this group, 1536 patients (48% males and 52% females) with positive skin prick tests to pollens and seasonal symptoms were selected. The mean age was 32 years (range 8–81 years).

All patients were skin tested with a battery of common aeroallergens at 50 HEP (Inmunotek, Madrid, Spain). The pollens included 25 representative species, based on pollen counts. Steel lancets (Allergy Pricker; DHS) were used. All skin test sites were evaluated after 20 min.

The area of each wheal was measured by planimetry using the Prick-Film system (Inmunotek). The skin prick test results were expressed in mm². A positive reaction was defined as a wheal of at least half the size of the histamine wheal in the absence of a reaction to the negative control.

Additionally, we also obtained the 'degree of atopy' for each patient by adding the wheal areas of the positive allergens tested. A Spearman rank correlation coefficient was used to correlate the degree of atopy with the prevalence of asthma.

Results: We observed a rhinitis prevalence of 93%, conjunctivitis 89% and asthma 41%. Sensitisation prevalence was as follows: grasses 87%, *Olea* 43%, *Chenopodium* 27%, *Platanus* 22%, *Cupressus* 13% and *Parietaria* 11%.

The prevalence of pollen polysensitisation was 75%.

Patients were divided into six groups according to their degree of atopy using the following ranges: 0–99 mm²; 100–199 mm²; 200–299 mm²; 300–399 mm²; 400–499 mm² and 500–599 mm². The prevalence of asthma within these ranges was: 37%; 38%; 40%; 46%, 48% and 55%, respectively (Spearman rank correlation $r_s = 0.96$ $P < 0.05$).

Conclusion: The prevalence of asthma within this pollinosis population in Spain, is significantly correlated with a greater degree of atopy.

526

Lymphocyte populations in patients with severe versus mild persistent atopic asthma

Tsybulkina, VN¹; Kurmaeva, N¹; Skibo, Y²; Abramova, Z²; Tsybulkin, N²; DuBuske, LM⁴

¹Kazan State Medical University, Kazan, Russia; ²Kazan Federal University, Kazan, Russia; ³Kazan State Medical Academy, Kazan, Russia; ⁴Immunology Research Institute of New England, Gardner, MA, United States

Background: Atopic asthma (AA) is a chronic inflammatory disease with heterogeneity of clinical course and severity perhaps related to different pathogenic mechanisms involved in generation of chronic airway inflammation.

Method: Patients 19 to 45 years old with persistent AA in remission were divided into two groups- mild (M-AA) and severe (S-AA) with moderate cases excluded. The control group (CG) consisted of healthy subjects. Groups of comparable age and gender and were 10 persons each. All subjects had skin tests, IgE levels, bronchoprovocation tests, lung function and blood counts with lymphocyte populations assessed by flow cytometry (FASCalibur, Becton Dickinson), identifying CD3, CD4, CD8, CD16, CD56 (MultiTEST IMK, Becton Dickinson).

Results: The clinical condition of patients in both AA groups was comparable, their lung function differed: FEV1 and FVC in S-AA patients 44.3310.56% and 57.507.20% of predicted vs 89.008.05% and 92.257.59% in M-AA group ($P < 0.05$). Mean absolute number of T cells (CD3+) in S-AA group (2473.97940.30) was less than in M-AA (3478.881427.29). Numbers of T helper cells (CD4+) were similar in all groups (1600.10628.10 in S-AA, 1770.15780.54 in

M-AA and 1704.50433.20 in CG), but their proportion was significantly greater in S-AA patients compared to M-AA (48.758.10% vs 36.758.28%, $P < 0.05$). Cytotoxic T lymphocytes (CTL) counts were less in S-AA (755.38241.44) compared to M-AA and CG (1516.85725.57 and 1302.83479.94 respectively) as were percentages which were 23.501.73% in S-AA vs 31.383.74% in M-AA and 30.335.68% in CG ($P < 0.05$). The CD4+/CD8+ ratio was nearly twice greater in S-AA (2.100.47) then in M-AA (1.190.33, $P < 0.05$), while CG group was also less t (1.390.32, $P < 0.05$).

Conclusion: Significant reduction of lung function in S-AA patients in remission indicates bronchial inflammation with an increased proportion of CD4+ cells and increased CD4+/CD8+ ratio suggesting a major role of CD4+ cells in maintaining inflammation in S-AA patients.

527

Nocturnal continuous positive airway pressure in severe asthma: a pilot study

D'Amato, M¹; Stanziola, AA¹; Radicella, D¹; Lanza, M¹; Vitale, C¹; Caccavo, G¹; Sofia, M¹

¹Federico II Napoli, I Clinica di Malattie Respiratorie, Napoli, Italy

Background: It has been reported that brief periods of nocturnal continuous positive airway pressure (CPAP) reduces airway reactivity in rabbits. This effect persists 12–24 h after its discontinuation (Xue Z. Et al. J Appl Physiol 2011;111:353–357). We evaluated short term effects of nocturnal CPAP in severe asthma.

Method: In this pilot study four nights CPAP was applied to 10 patients (5M, 5F mean age 50.9 (range 49–56 years) with severe asthma. The patients were on regular treatment with LABA + inhaled corticosteroids (≥ 1000 mg fluticasone/day) and 5 of them with anti IgE treatment (Omalizumab). Five patients were on systemic corticosteroid treatment (Prednisone mean daily dose 17 mg [Range 10–30 mg]). All patients at polysomnography did not reveal any significant breathing sleep disorder. CPAP was titrated in autotitrating with values between 4 and 20 cmH₂O (mean value = 6). Asthma control test (ACT) baseline and after a month was measured and daily peak flow (morning and evening recorded) was measured from 2 weeks before baseline to 2 weeks after the treatment. PEF variability for each patient was calculated as PEF amplitude% over 24 h periods, amplitude% mean, defined as (highest PEF-lowest PEF)/(mean value of two) X100%, averaged over 1 week.

Results: Two patients were unable to tolerate nocturnal CPAP. In eight patients baseline PEF amplitude resulted $37.6 \pm 2.8\%$; it significantly decreased during nocturnal CPAP (nCPAP) period to $26.5 \pm 3.5\%$ and in the week after nCPAP discontinuation (30 ± 2.9 ($P < 0.05$ t test improve). The baseline ACT was 11 (range 8–15); after 1 month it increased to 21 (range 15–25).

Conclusions: Brief period of nocturnal CPAP reduce PEF variability and improve control in severe asthma at a short term evaluation. Larger and longer studies are required to evaluate this kind of intervention in severe asthma.

528

Silent gastroesophageal reflux disease is frequent in children with uncontrolled asthma but without clinical importance

Jedynak-Wasowicz, U¹; Glodzik, I¹; Cichocka-Jaroszc, E¹; Lis, G¹

¹Chair of Pediatrics, Department of Pulmonology, Allergy and Dermatology, Jagiellonian University Medical College, Krakow, Poland

Background: Bronchial asthma and gastroesophageal reflux disease (GERD) may coexist together. Also GERD may be implicated in the recurrent respiratory tract infections in children. The uncontrolled asthma, infections and gastroesophageal reflux may overlap and have the same clinical presentation.

The aim of the study was to assess:

- 1 the prevalence of GERD in asthmatic children without clinical symptoms of GERD and in children with recurrent respiratory tract infections;
- 2 the implication of the reflux episodes (RE) in developing of respiratory symptoms in both studied groups.

Method: Thirty-eight children with uncontrolled asthma (27 boys, mean age 14.4 ± 3.2 years) and 24 children with recurrent infections of respiratory tract as control group (15 boys, mean age 13.6 ± 2.2 years) were enrolled into the study. Twenty four-hour monitoring of multichannel intraluminal impedance (MII) with pH-metry was performed in all patients. Children were asked to report any episodes of cough, dyspnea or any respiratory distress, hoarseness, abdominal or chest pain. The clinical exercise testing was done during pH-impedance monitoring also. The clinical symptoms and records of pH-impedance probe were analysed before and after exercise testing. Children with recurrent infections were healthy at the day of study. Asthma treatment was continued during the investigation in all asthmatic children.

Results: Acidic GER was diagnosed in 15 (39.5%) asthmatic children and in six children (25%) of control group. There were no significant difference in any of analysed MII parameter between both groups. The exercise did not increase reflux episodes during and 30 min after testing compare to pH-impedance probe record 30 min before testing and it did not significantly predispose to any of analysed clinical symptom. Cough was the most common reported symptom in both groups (asthma group – 44.7% vs control group – 37.5%). But we did not find correlation between cough and episodes of proximal or distal reflux in asthmatic children with acidic GER (respectively $Rho = 0.05$ and $Rho = 0.08$).

We detected the slight increase in GER events in LABA-treated asthmatic children ($P = 0.08$).

Conclusion:

- 1 Silent acidic GER is frequent in children with uncontrolled asthma.
- 2 Gastroesophageal reflux episodes were not relevant to any respiratory symptoms in studied groups.
- 3 Short-lasting intensive exercise did not induce reflux episodes.

529

The roles of vitamin D and cathelicidin in the development of acute asthma attacks in children

Batmaz, SB¹; Arikoglu, T¹; Karaismailoglu, E²; Kuyucu, S¹

¹Department of Pediatric Allergy and Immunology, Mersin University Faculty of Medicine, Mersin, Turkey;

²Department of Biostatistics, Hacettepe University Faculty of Medicine, Ankara, Turkey

Introduction: Recent evidence, about the various effects of vitamin D (vit D) on innate and adaptive immunity has led to search for its role in asthma and allergic diseases. The defects in the innate immune system, such as the capacity to increase the production of broad spectrum antimicrobial peptides like cathelicidin may predispose to infectious complications. The aim of this study was to determine the role of vit D and cathelicidin in the development of acute asthma attacks among 7–17 years old allergic asthmatic-children.

Material and Methods: The study included 35 patients with acute asthma exacerbation triggered by an infection, 32 children with controlled asthma and 21 healthy children, all matched by sampling season and for asthma subgroups, matched by mono-mite sensitisation and previous severity and medication score of asthma. In all groups, a comprehensive questionnaire, serum 25-OH vit D, vitamin D-binding protein (VDBP) and cathelicidin levels, markers of allergy, viral serology and spirometric indi-

ces were employed. Factors that influence serum vit D levels and the development of asthma attacks were evaluated with multivariate linear and logistic analysis.

Results: The mean serum vit D level was 14.09 ± 5.75 in the attack group, 28.47 ± 13.88 in the stable asthma group and 12.95 ± 7.15 in healthy controls. The differences between attack and stable asthma groups were highly significant ($P < 0.001$). On the contrary, mean cathelicidin level was significantly higher in acute asthma group than controlled asthmatics ($P = 0.002$). Cathelicidin levels showed a negative correlation with vit D levels ($P = 0.002$, -0.380 spearman c.c). Furthermore, there was a positive correlation between the spirometric indices and the level of vit D among asthmatics ($P \leq 0.05$). Multivariate analysis of risk factors that may influence vit D levels revealed that younger age ($P = 0.046$), high BMI ($P = 0.025$), longer duration of sun exposure ($P \leq 0.001$), and high amount of dietary vit D ($P \leq 0.001$) independently increased serum vit D levels. Furthermore, multivariate analysis of risk factors that may result in acute asthma vs controlled asthmatics showed that the increase in serum levels of vitamin D significantly reduced the risk of asthma attacks ($P = 0.030$, adjusted odds ratio 0.862) independent of age, sex, allergic markers, use of inhaled steroids, BMI, time spent outside (as a marker of activity level), serum levels of cathelicidin and VDBP.

530

Age-related changes in airway resistance using an impulse oscillation system in patients with asthma

Iwanaga, T¹; Kume, H¹; Okimoto, N¹; Watatani, N¹; Imbe, S¹; Miyajima, H¹; Santo, H¹; Sato, R¹; Nishiyama, O¹; Sano, H¹; Higashimoto, Y¹; Nakajima, H¹; Tohda, Y¹
¹Respiratory Medicine and Allergology, Kinki University Faculty of Medicine, Osakasayama, Japan

Background: The characteristics of elderly asthma include decreased pulmonary function, and in particular, airflow obstruction. In the daily care of asthma, the degree of airflow obstruction as measured through spirometry is used as an index of asthma control, but because the implementation of spirometry requires the maximal forced expiration of the subject, this examination is a burden for elderly subjects. Impulse oscillation system (IOS), which began to be applied clinically in recent years, is able to measure airway resistance under quiet breathing using impulse waves, and is also able to separate airway resistance into central airway components and small airway components through frequency analysis.

IOS is therefore useful for analyzing airflow obstruction in elderly or pediatric subjects; however, the usefulness of evaluating airway resistance in elderly asthmatics using IOS is not clear. In this study, we investigated the usefulness of IOS by measuring airway resistance and the correlation between IOS and spirometry in elderly patients with asthma, and also to compare the results with those of non-elderly patients with asthma.

Method: IOS, spirometry and flow volume curve were performed for 51 elderly (65 years old or older) and 58 non-elderly (below 65 years old) patients with asthma.

Results: The correlation between lung function and IOS, there were significant correlation between IOS parameters (R5, R5-R20, Fres, X5) and lung function [% FEV₁ (forced expiratory volume in 1-s), % Vdot50, %Vdot25, %MMF (maximal mid-expiratory flow)] in each group. The correlations between age and IOS parameters: R5, R5-R20, X5 and Fres were significantly correlated with age, with Fres showing the strongest association. In the elderly group, R5 was significantly higher than the non-elderly group (0.30 vs 0.40, $P < 0.01$). Significantly higher R5-R20 was exhibited in the elderly group compared with the non-elderly group (0.04 vs 0.10, $P < 0.01$). X5 showed significantly low value in the elderly group than in the non-elderly group (-0.11 vs -0.15 , $P < 0.05$). Fres was a significant higher in the elderly group than in the non-elderly group (11.92 vs 15.83, $P < 0.05$).

Conclusion: These results suggest that IOS can be used to reveal small airway lesions in elderly patients with asthma and is useful as a method of evaluating airway lesions in patients with asthma.

531

Lung ventilation and capnometry in patients with uncontrolled bronchial asthma

Iashyna, L¹; Feshchenko, Y¹; Ishchuk, S¹

¹State Organization 'National Institute of Physiology and Pulmonology named after FG Yanovsky National Academy of Medical Sciences of Ukraine', Kiev, Ukraine

Background: Gas exchange abnormalities are the dangerous complication of uncontrolled BA. We investigated the relationship between lung ventilation and capnometry indices in patients with uncontrolled BA.

This study aimed to investigate capnometry indices in patients with uncontrolled BA compare to healthy subjects.

Method: Bodyplethysmography, capnometry. Data are presented as mean \pm SD.

Results: A total of 30 subjects (age 51.6 ± 2.8 years; 39% male) were enrolled: BA group ($n = 15$, mean%FEV₁ = 72.9%, ACT = 12.9), control group ($n = 15$, mean%FEV₁ = 99.1%). All subjects were performed bodyplethysmography and capnometry, we compared the results between groups.

All bodyplethysmography and capnometry indices of BA subjects were significantly ($P < 0.05$) different from control. The mean values Rtot,% was 161.8 ± 16.1 and 99.6 ± 7.8 , ITGV,% was 109.9 ± 5.8 and 91.7 ± 4.0 , RV,% was 131.6 ± 8.8 and 102.6 ± 4.7 in BA and control group respectively. The capnometry results: PeCO₂, kPa (partial pressure CO₂ in expired air) was 2.7 ± 0.1 and 3.3 ± 0.1 , FeCO₂,% (fraction CO₂ in expired air) was 2.9 ± 0.1 and 3.5 ± 0.1 , PetCO₂, kPa (partial pressure of end-tidal CO₂) was 4.1 ± 0.1 and 4.8 ± 0.1 , FetCO₂,% (fraction of end-tidal CO₂) was 4.4 ± 0.1 and 5.1 ± 0.1 in BA and control group respectively.

Conclusion: In uncontrolled BA patients CO₂ removal from the organism reduced with degree of lung hyperinflation increasing. Thus capnometry might be a useful tool to detect the respiratory failure in BA patients.

533

Comparison of the bronchial sensitivity and reactivity using impulse oscillometry system between cough-variant asthma and classic asthma in pre-school children

Jungwon, Y¹; Hyeyoung, H¹; Hyemi, J¹; Manyong, H¹
¹Pediatrics, CHA Medical Center, Seongnam, Korea

Background: Little data is currently available on the use of the impulse oscillometry (IOS) parameter in analyzing the lung function of pre-school children with cough-variant asthma (CVA) and classic asthma. The aims of this study were to compare the bronchial sensitivity and bronchial reactivity between patients with CVA and those with classic asthma by IOS, and to investigate if the IOS parameters can distinguish one group from the other.

Method: A methacholine challenge test and a pulmonary function test were performed in 43 children with classic asthma and 28 children with CVA, using IOS, and the respiratory resistance (Rrs) and reactance (Xrs) were obtained. The bronchial sensitivity of all subjects was assessed using methacholine provocative concentration causing a 80% increase in reactance at 5 Hz (PC_{80-Xrs5}) and the concentration with Z scores of reactance at 5 Hz going beyond 0.2 values (Zs_{Xrs5}). The bronchial reactivity was calculated from the degree

of dose-response slope (DRS) for airway resistance and reactance.

Results: There was no significant difference in bronchial sensitivity between the two groups. However, the classic asthma group showed a significantly higher bronchial reactivity in reactance at 5 Hz (DRS_{Xrs5}) compared with the CVA group ($P = 0.044$).

Conclusion: DRS_{Xrs5} is suggested to be a useful marker in distinguishing CVA from classic asthma through methacholine challenge testing using IOS in pre-school children.

535

Impact of sputum cells profile and inflammatory markers on airway responsiveness and asthma severity in different asthma phenotypes in children

Nakonechna, A¹; Antipkin, V²; Umanets, T²; Lapshyn, V²; Zadorozhnaja, T²; Pustovalova, O²
¹Immunology, Hull and East Yorkshire Hospitals NHS Trust, Neston, United Kingdom; ²Obstetrics and Gynaecology, Institute of Paediatrics, Kiev, Ukraine

Background: Heterogeneity in asthma is recognised by different inflammatory cell patterns in airway secretions. However, the role of sputum cells and inflammatory markers in airway responsiveness in different childhood asthma phenotypes is still unclear.

Objective: Investigate whether the sputum cell profile and expression of inflammatory markers impact on airway responsiveness and asthma severity in children with different asthma phenotypes.

Method: Seventy eight children aged 6–12 years with asthma and 25 age-matched healthy controls were assessed including: skin prick testing (SPT), lung function, total and antigen specific IgE, induced sputum analysis. Expression of CD4+, CD68+, IgE, TNF-alpha and matrix metalloproteinase-1 (MMP-1) in sputum cells were assessed using immunocytochemistry. Multivariate regression was used to determine whether sputum cell profile, airway responsiveness and disease severity varied between phenotypes.

Results: Among investigated children 69.2% had atopic asthma with increased total and specific IgE levels, positive SPT at least to one allergen. They had mild-to-moderate asthma (87%) and induced sputum eosinophilia along with increased level of sputum lymphocytes, mast cells and bronchial epithelial desquamation. Airway hyperresponsiveness strongly associated to higher level of eosinophils, highly elevated expression of CD4+, IgE and CD68+, especially in moderate asthma ($P < 0.05$). In contrast 30.8% children with non-atopic asthma had moderate-to-severe asthma,

induced sputum neutrophilia and more significant airway hyperreactivity in metacholine and exercise-induced tests, that both correlated with high expression of CD68+, MMP-1, TNF-alpha and asthma severity ($P < 0.05$).

Conclusion: Sputum cell profile, inflammatory markers and airway responsiveness vary in different asthma phenotypes in children. These findings suggest a role of inflammatory markers (cells and receptors) in the phenotypic classification of asthma and determining the severity of disease and therapy in childhood asthma management.

536

The role of Cocksackie and adenoviral receptor in TNF alpha driven inflammation in lung epithelial cells

Hicks, AP¹; Morton, P¹; Parsons, M²; Santis, G¹
¹Asthma, Allergy & Lung Biology, Kings College London, London, United Kingdom; ²Randall Division of Cell and Molecular Biophysics, Kings College London, London, United Kingdom

Background: The regulation and maintenance of an intact epithelial cell layer is crucial to the homeostasis of many different tissues including the respiratory tract. One of its key functions is the creation of a physical barrier from the external environment. This is achieved through the combination of adherens and tight junctions, known as the apical junctional complex (AJC). In asthmatic patients there is evidence of AJC disruption leading to exposure of underlying dendritic cells and consequently a greater risk of inflammatory response. Cocksackie and adenoviral receptor (CAR) is an important component in these junctional complexes. Its role in viral infection is well characterised, however, its physiological role in cell-cell junction stability and function is poorly understood.

Method: We have set out to identify whether CAR influences cell-cell junction stability in response to inflammatory cytokines. Human bronchial epithelial cells (HBEC) expressing either CARGFP (CARGFP) or a CAR specific shRNA (CARKD) were used to develop an epithelial tissue equivalent organotypic model in which cells are grown on top of a collagen gel containing fibroblasts over 10–14 days. Initial experiments were performed probing immunoprecipitated CAR with phosphotyrosine or phospho-ser/thr antibodies to detect CAR phosphorylation downstream of TNF. PKCδ expression was silenced using siRNA and levels of CAR phosphorylation and PKCδ were measured using western blotting. Using a tissue equivalent epithelial organotypic model allowed us to

study the effect of CAR expression levels on epithelial morphology in a physiologically appropriate environment.

Results: Using this model we have observed dramatic changes in the structure of the epithelium in CARKD but not CARGFP cells. Moreover, biochemical analysis demonstrates that CAR undergoes phosphorylation within the cytoplasmic domain in the presence of TNF alpha in a manner dependent upon PKC δ . We are developing CAR phospho-specific antibodies to enable us to determine the subcellular localisation of this modified species of CAR.

Conclusions: Our data shows that CAR is phosphorylated downstream of TNF, a known mediator of inflammation in the lung. This suggests a possible role for CAR in the control of epithelial responses to external inflammatory cues. Future experiments will be aimed at analysing the role of phosphorylated CAR in mediating trans-epithelial-migration of inflammatory cells, and the presence of phospho-CAR in tissues of asthma patients.

537

Lipopolysaccharide, Der p1 but not Fel d1 induce expression of selected phospholipases A₂ (PLA₂s) in peripheral blood mononuclear cells of asthmatics

Pniewska, E¹; Sokolowska, M^{1,2}; Kacprzak, D¹; Pawliczak, R¹

¹Department of Immunopathology, Medical University of Lodz, Lodz, Poland; ²Critical Care Medicine, National Institute of Health (NIH), Bethesda, MD, United States

Background: LPS and allergens affect the initiation, course and exacerbation of asthma. They can activate many intracellular signaling pathways. The phospholipase A₂ superfamily consists of 30 enzymes responsible for phospholipid hydrolysis. The involvement of PLA₂s in inflammatory diseases relates to their enzymatic activity reflected by their participation in production of broad range of lipid inflammatory mediators and their non-enzymatic properties focused on regulation of immunocompetent cells functions. Secretory and cytosolic PLA₂s are thought to have the major role in asthma pathogenesis.

Aim: The aim of the study was to investigate the changes in expression level of selected PLA₂s genes (*PLA2G2A*, *PLA2G4A*, *PLA2G5*, *PLA2G6*, *PLA2G7*, *PLA2G10*, *PLA2G15*, *PAFAH1B1*) in PBMCs of asthmatics and healthy controls after stimulation with LPS, recombinant Der p1 and Fel d1.

Method: Four patients with severe asthma and four healthy subjects were enrolled to the study. The asthmatics were randomly selected from patients treated in the

Department of Internal Medicine, Asthma and Allergy of Medical University of Lodz. Peripheral blood mononuclear cells (PBMCs) (2×10^5) isolated from blood of asthmatics and healthy subjects were stimulated with rDer p1 (1 μ g/ml), rFel d1 (1.25 μ g/ml) for 6 days and LPS (100 μ g/ml) for 8 h. The control cells from the same subjects were incubated in the same conditions without these factors. After cell stimulation total RNA was isolated. The cDNA prepared from 400 ng of RNA was used with TaqMan Low Density Arrays (LDA). The changes in expression of 8 PLA₂s genes involved in asthma pathogenesis were calculated by usage of $2^{-\Delta\Delta C_t}$ method.

Results: Expression of three studied PLA₂s genes (*PLA2G4A*, *PLA2G6*, *PLA2G15*) were significantly changed in PBMCs after stimulation with rDer p1 between patients and healthy subjects. Only *PLA2G15* gene was expressed at significantly different level after stimulation with LPS. Fel d1 did not influence the expression of studied PLA₂s genes.

Conclusion: LPS and Derp1 can influence expression of selected PLA₂s genes in PBMCs of asthmatics in comparison to healthy volunteers. Der p1 seems to be the most potent stimulator of PLA₂s expression.

Funded by Polish National Science Center grant N N402 516939.

538

The alveolar parenchyma is subjected to a mast cell associated tissue inflammation and remodeling in patients with uncontrolled allergic asthma, but not in controlled asthmatic patients

Andersson, CK¹; Weitoft-Lundström, M²; Bergqvist, A¹; Westergren-Thorsson, G²; Björner, L¹; Erjefält, JS²

¹Department of Respiratory Medicine & Allergology, Lund University, Lund, Sweden; ²Department of Experimental Medical Science, Lund University, Lund, Sweden

Background: A significant proportion of patients with allergic asthma have persistent symptoms despite treatment with inhaled steroids. Little is however known regarding the peripheral inflammatory components in asthmatic patients. Our aim was to study the phenotype of alveolar mast cells, and the connection to tissue remodelling and inflammation, in patients with controlled and uncontrolled asthma (treated with corresponding inhaled steroid dose) compared to non-atopic healthy controls.

Methods: Bronchial and transbronchial biopsies from non-atopic controls ($n = 8$), patients with controlled ($n = 10$) and uncontrolled ($n = 16$) asthma were pro-

cessed for immunohistochemical identification of mast cell subtypes and expression of related mediators. Mast cell alteration in relation to tissue remodelling and Th2 inflammation in peripheral lung was studied as well.

Results: Changes in mast cell densities were not observed in the alveolar parenchyma in patients with controlled asthma compared to healthy controls. However, in the alveolar parenchyma in uncontrolled asthmatics an increase in densities of both MC_{TC} ($P = 0.05$) and MC_T ($P = 0.003$) compared to controls was found. The expression of the high affinity IgE receptor (Fc ϵ RI) as well as IgE bound to alveolar mast cells was increased in uncontrolled asthma compared to healthy controls ($P < 0.001$). Changes in peripheral Th2 inflammation was observed in uncontrolled asthmatics, but not in controlled asthma, compared to controls. Increased densities of collagen were observed in the alveolar parenchyma in patients with uncontrolled asthma ($P = 0.007$) but not in controlled asthmatics ($P = 0.7$) compared to controls. The increased number of alveolar MC_{TC} in the uncontrolled asthmatics was correlated to the density of collagen in the alveolar parenchyma ($r_s = 0.71$, $P = 0.03$).

Conclusions: Our data suggest that steroid treated uncontrolled atopic asthmatics, but not patients with controlled asthma, have an altered mast cell phenotype in the alveolar parenchyma. These alterations are closely connected to alveolar tissue remodeling and Th2 inflammation. This reflects an important involvement of mast cells in development of inflammation in the alveolar parenchyma in the uncontrolled asthma response and underscores the need to target peripheral lung inflammation in this asthma phenotype.

539

Role of exhaled breath temperature in the monitoring of airways inflammation in children

Barberi, S¹; D'Auria, E¹; Poli, P¹; Di Vito, M¹; Gualdi, C¹; Giovannini, M¹

¹Department of Pediatrics, San Paolo Hospital, University of Studies of Milan, Milan, Italy

Background: The aim of the study is to measure the temperature of the exhaled breath (EBT) in asthmatic, rhinitic and healthy children, assessing the potential correlation between EBT and the individual's clinical conditions, and to evaluate whether the EBT is an effective means of monitoring patients with uncontrolled asthma.

Method: Three-hundred subjects have been enrolled (M: 194; F: 106; 150 asthmatic, 98

rhinitis and 52 healthy controls; age: 4–18 years). All children have been visited and to children's parents it has been asked to answer to two questionnaires polarised on respiratory symptoms, previous therapies and on exposition to tobacco smoke. Spirometry and Skin Prick Tests were also performed.

Results: Fifty-two of the 300 individuals enrolled represented the control group; in this group average EBT values were lower (M: 29.71 ± 1.04 ; F: 29.42 ± 0.99) compared to the values found both among the group of rhinitic (M: 31.03 ± 2.09 ; F: 31.11 ± 2.20) and the group of asthmatic children (M: 32.55 ± 1.83 ; F: 32.83 ± 1.89). EBT values were partially overlapping in asthmatic and rhinitic individuals but it was possible to highlight a significant difference between the average values of the EBT in both categories, according to the symptoms shown at the moment of measurement and according to the symptoms control. In fact, an increase of such values in the sub-group of asthmatic was useful to differentiate asthmatic patients with episodes of moderate-serious dyspnea in the last period from those asymptomatic or with mild symptoms, according to clinical history, clinical examination and the answers to the survey filled by parents. Similarly, within the category of rhinitic individuals, we observed an increase of values in symptomatic child (nasal obstruction, rhinitis) at the time of measurement.

Conclusion: The non-invasive monitoring of EBT is a valid inflammation marker, in particular to discriminate, among asth-

matic and rhinitic individuals, those with a good control compared to those poorly controlled, and to assess compliance with the ongoing therapy and the development of the pathology. EBT could therefore be useful to monitor asthmatic children: it can help to evaluate the seriousness of the disease and the response to treatment, particularly during the worsening phase of the disease. However, there are still limits about reproducibility, variability and sensitivity. Further studies are necessary to evaluate the importance of measuring the EBT of each patient in their follow up.

540

Role of gastro esophageal reflux disease in pulmonary fibroblast differentiation

Chiang, C-C¹; Shih, WA¹; Cheng, CM¹

¹Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung, Taiwan

Background: GERD (gastro esophageal reflux disease) is a reflux disease that gastric content regurgitate into esophagus. Epidemic studies indicated that micro aspiration of gastric fluid in GERD patients is associated with the prevalence of chronic respiratory diseases. Inflammation in the context of airway residential cells is now generally recognised to be involved in the immune modulation. We had previously demonstrated that gastric fluid acts as an inflammatory mediator in macrophages and airway smooth muscle cells. In the present study, we hypothesised that, in the context of pulmonary residential cells,

micro aspiration of gastric fluid induces inflammatory response and initiates the fibroblast to myofibroblast differentiation.

Method and Results: Factors involved in fibroblast differentiation were analyzed. Using human normal bronchial epithelial cells (NL-20), the inflammatory cytokine and chemokine expression were assessed by a cytokine array analysis. The normal pulmonary fibroblast cells (HFL-1) was used to evaluate the fibrotic progression induced by gastric fluid. Our data showed that, gastric fluid treatment induced inflammatory cytokines and chemokines, such as eotaxin, IGFBP-1, IGFBP-4, IL-5, IL-6, MCP-2, MCP-3 and PDGF-BB expression in pulmonary epithelial cells. Condition medium collected from pulmonary epithelial cells and macrophage cells induced migration and α -SMA protein expression in pulmonary fibroblast cells. By using RT-PCR and Elisa assay, we also observed that IL-6, IL-8 and transforming growth factor-beta (TGF- β) were enhanced in pulmonary fibroblast cells by gastric fluid and condition medium treatment.

Conclusion: In summary, our current data indicated that, gastric fluid alone or the poor mixture of proinflammatory mediators induced by gastric fluid in the venue of pulmonary context may cause the pulmonary fibroblast cell inflammation, migration, differentiation, suggesting that chronic inflammation induced by gastric fluid aspiration in pulmonary residential cells has a causal relationship in the pulmonary fibroblast differentiation and the subsequent pulmonary fibrosis.

Poster Session 9

Understanding the worldwide implications of the asthma epidemic

541

Clinical characteristics of the elderly asthma

Park, H-K¹; Cho, E-J¹; Lee, S-E¹; Mok, J-H¹; Kim, M-H¹; Cho, W-H¹; Lee, K¹; Kim, K-U¹; Jeong, Y-Y²; Jeon, D-S¹; Kim, Y-S¹; Lee, M-K¹

¹Internal Medicine, Pusan National University School of Medicine, Busan, Korea; ²Internal Medicine, Gyeongsang National University School of Medicine, Jinju, Korea

Background: The prevalence of asthma has increased annually, and the number of elderly asthmatics has increased. It has been well known that the prevalence of depression and anxiety is higher in the asthma patients than general population. In this study, we evaluated severity of asthma, comorbidities, degree of asthma control in the elderly asthma patients. We also evaluated whether there is any difference in depression and anxiety depending on the degree of control of asthma in the elderly asthma patients.

Method: Total of 280 patients with asthma who had been treated more than 1 year was enrolled. The elderly asthma was defined as being more than 65 years old. Demographic data, anxiety and depression scores as well as the level of asthma control were evaluated. Patient-reported depressive symptoms, anxiety were evaluated using Beck Depression Inventory (BDI), the State-Trait Anxiety Inventory (STAI).

Results: There was no difference in the severity or degree of control of asthma between elderly and younger asthma patients. Hypertension was significantly more prevalent in elderly asthma patients. Elderly asthma patients reported more depressive symptoms than younger asthma patients and healthy controls ($P = 0.013$). Anxiety score was higher in the younger patients (STAI-X1; $P < 0.001$, STAI-X2; $P = 0.036$). There was no difference regarding anxiety between elderly asthma patients and the elderly healthy controls.

Conclusion: The elderly asthma patients had higher degree of depression than younger asthma patients. Therefore, it would improve the prognosis of the disease to assess and treat depression early in the elderly asthma patients.

542

Remission in adult onset asthma and contributing factors

Celebi Sozener, Z¹; Aydin, O¹; Mungan, D¹; Misirligil, Z¹

¹Department of Pulmonary Diseases, Division of Immunology and Allergy, Ankara University School of Medicine, Ankara, Turkey

Background: The studies on the natural history of adult onset asthma and the contributing factors for its prognosis are few in the literature. The aim of this study is to review adult onset asthma patients; their remission status and the contributing factors.

Method: Files of 168 asthma patients diagnosed in 2006 were evaluated. Among them, 124 were contacted in 2012. The patients were reviewed according to demographic data, treatment compliance, PFTs, clinical symptoms and asthma control test. Patients who were in complete control of asthma without any medication for the last 2 year were classified as in remission. Factors affecting the remission were investigated.

Results: The study group consisted of 124 patients (113F, 11M) were between 22 and 77 years of age. Seventy percent had a history of asthma before 40 years of age. Atopy ratio was 48%. Non compliance rate was 23%. Thirteen percent had uncontrolled disease, 21% had partial control and 64% had complete control. Remission was observed in 8.87% (n:11) of the total group. Median age was 42 years and the age of asthma onset was 33 years in patients with remission. Among this group; 8 had atopy, 7 had family history of asthma and 7 had allergic rhinitis. They all had been using inhaled corticosteroids with a better compliance rate (64%).

Conclusion: According to 5 year follow up data, the remission rate of asthma was 8.87% in our study group. Patients with atopy, allergic rhinitis, early diagnosis and good compliance seemed to have higher chances of remission.

543

Gender-specific impact of rhinitis on eosinophilic airway inflammation in adults with mild-to-moderate asthma

Imaoka, M¹; Kishikawa, R¹; Shimoda, T¹; Iwanaga, T¹

¹Department of Internal Medicine, National Hospital Organisation Fukuoka National Hospital, Fukuoka, Japan

Background: The majority of patients with asthma have a history or evidence of rhinitis, and rhinitis is associated with increased asthma severity; potential gender specific differences in the pathophysiology and clinical presentation of both asthma and rhinitis have been shown. The aim of this study was to investigate the gender-specific impact of rhinitis on eosinophilic airway inflammation in steroid-naïve adults with mild-to-moderate asthma.

Method: The subjects comprised 269 Japanese asthmatic adults [100 men and 169 women, median (range) age 53 (20–90) years, 79% with rhinitis] with mild-to-moderate asthma who were untreated with glucocorticosteroids and during attack-free periods. We used the levels of fractional exhaled nitric oxide (FeNO) as a marker of eosinophilic airway inflammation. The FeNO concentration was measured using the recommended online method. We retrospectively compared the levels of FeNO between asthmatic patients with and without rhinitis, separately for men and women.

Results: In 100 men, 85 asthmatic patients with rhinitis had significantly higher FeNO levels compared with 15 asthmatic patients without rhinitis (62.7 ± 75.9 vs 42.4 ± 23.4 ppb, respectively; $P = 0.03$); in 169 women, there was no significant difference in FeNO levels between 128 asthmatic patients with rhinitis and 41 asthmatic patients without rhinitis (39.6 ± 35.0 vs 41.7 ± 44.7 ppb, respectively; $P = 0.39$).

Conclusion: Our results suggest that rhinitis significantly increases eosinophilic airway inflammation in men but not in women with mild-to-moderate asthma. Other inflammatory cells than eosinophils alone may play a major role in the patho-

genesis in women with both asthma and rhinitis.

544

The change of the prevalence of asthma and its association with rhinitis and smoking in a survey of Japanese adults

Okada, C¹; Tanimoto, Y²; Horiguchi, H¹; Fushimi, K³
¹Department of Medicine of the Headquarter, National Hospital Organization, Tokyo, Japan; ²Department of Hematology, Oncology, Allergy and Respiratory Medicine, Okayama University Graduate School of Medicine, Okayama, Japan; ³Tokyo Medical and Dental University, Tokyo, Japan

Background: In Japan, the study about the prevalence of asthma and its change in adults was very few, compared to the studies in children. So, we investigated the prevalence of asthma in adults and its change in same area in Japan. And we also investigate the risk factors which influence the prevalence of asthma.

Method: The studies were performed in same area in 2006 and 2011 with same process using the ECRHS questionnaire, to examine the prevalence of asthma. Two thousand and four hundred people, who were chosen with randomised procedure from the citizen of Kurashiki-city (population is about 470 thousand) in Japan, were enrolled to these studies.

Results: The prevalence of asthma in 20–44 years old adults is 9.1% (male 8.9%, female 9.3%) in 2006 and 11.4% (male 12.5%, female 10.6%) in 2011. So, the prevalence of asthma increased in 20–44 years old adults after 5 years. But the prevalence of asthma decreased in 20–85 years old adults from 9.5% to 7.9%. About the risk factors of asthma, the result of the multivariate logistic regression analysis for association of rhinitis and asthma is OR = 2.03 ($P < 0.001$), and smoking and asthma is OR = 2.15 ($P < 0.001$) using 2006 data. And the prevalence of current smoker in 20–85 years old adults widely decreased from 60.9% in 2006 to 46.2% in 2011, in spite of the prevalence of rhinitis increased from 35% to 38.3%. These results may suggest that the decrease of current smokers as a risk factor influence the decrease of the prevalence of asthma.

Conclusion: The prevalence of asthma in 20–44 years old adults increased after 5 years, 2006 to 2011, but decreased in 20–85 years old adults. This result may indicate that the prevalence of asthma in elderly adults decreased. And the fact that the current smokers decreased in 5 years may influence this decrease. But, further examination of other factors, the change of medication etc. is necessary to confirm this influence.

545

Asthma severity and comorbid conditions among elderly patients

Wardzyńska, A¹; Kubsik, B²; Kosiński, S¹; Bieńkiewicz, B¹; Cieślak, M¹; Lewandowska-Polak, A¹; Makowska, J¹; Kurowski, M¹; Jędrzejczak-Czechowicz, M¹; Smorawska, E¹; Żaromińska, D¹; Kowalski, M¹

¹Department of Clinical Immunology, Rheumatology and Allergy, Medical University of Lodz, Lodz, Poland; ²Public Health Care Centre, Gostynin, Poland

Background: The presence of comorbid conditions may be related to asthma control and severity, especially in elderly patients.

Goal: The aim of this study was to evaluate the pattern of comorbidities and its impact on elderly patients with asthma.

Method: Patients with asthma: 93 elderly (above 65 years of age) and 78 younger (30–50 years old) were randomly selected from a group of 1700 asthmatics at the University Hospital Asthma Clinic database. Evaluation consisted of the questionnaire, Asthma Control Test (ACT), spirometry and SPT testing. Asthma control was assessed based on the ACT and GINA guidelines, asthma severity defined using ATS workshop 2000 criteria.

Results: Overall asthma severity and asthma control (including number of exacerbations over last 12 months) were similar in both groups. Elderly asthmatics had lower incidence of atopy compared to younger subjects (35.8% vs 72.3%, $P < 0.001$). The average number of diagnosed comorbidities was higher in elderly asthma patients than in younger group (8.4 vs 4.7, $P < 0.001$). Elderly patients as compared to younger had a higher incidence of osteoarthritis (64.5% vs 25.6%, $P < 0.001$), hypertension (64.5% vs 17.9%, $P < 0.001$) and coronary heart disease (52.7% vs 3.8%, $P < 0.001$). The average number of prescribed medicines was higher in elderly than in control group (7.4 vs 4.5, $P < 0.001$). 87.1% of elderly asthmatics and 44.9% of younger took more than five medicines simultaneously ($P < 0.001$). Prevalence of allergic rhinitis was lower in elderly asthma patients as compare to younger (33.3% vs 69.2%, $P < 0.001$), but severity of allergic rhinitis (assessed according to ARIA 2008 guidelines) in both groups was comparable. The prevalence of symptoms related to sinusitis as well as diagnosis of sinusitis, nonallergic rhinitis and nasal polyps were similar in elderly and younger asthmatics. There was no significant correlation between the presence of doctor-diagnosed commorbid conditions and asthma severity and control in the older group.

Conclusion: In the elderly patients with asthma treated by specialists the disease control and severity may be similar to

younger asthmatics, and are not related to the presence of comorbidities.

546

Ascaris sensitisation is associated with asthma severity in an urban population of the tropics

Buendía, E¹; Zakzuk, J^{2,3}; Mercado, D^{1,3}; Mantilla, A¹; Caraballo, L^{1,3}

¹Institute for Immunological Research, University of Cartagena, Cartagena, Colombia; ²University of Cartagena, Cartagena, Colombia; ³Fundemeb Foundation for the Development of Medical and Biological Sciences, Cartagena, Colombia

Background: Influence of *Ascaris* on asthma is not clear. Information about its role on disease severity is scarce and controversial. We evaluated the influence of *Ascaris* infection (ascariasis) and sensitisation on asthma severity in a group of patients living in an urban area of the tropics.

Method: Asthmatic patients ($n = 312$) living in the poorest communes of Cartagena, Colombia were recruited. Informed consent was obtained. Clinical assessment included questionnaires, allergy skin testing, spirometry, parasite stool examination and IgE serology. Asthma was defined according to the GINA criteria. Total and specific IgE to the extracts *Ascaris*, *Blomia tropicalis* (Bt) and *Dermatophagoides pteronyssinus* (Dp) and to the recombinant allergens Asc 1 3 and Asc s 1 were determined by ImmunoCap and ELISA, respectively. Relationships among *Ascaris* sensitisation and measures of asthma severity (severe dyspnea, dyspnea with physical activity, lung function parameters, nocturnal symptoms, oral corticosteroid use and asthma exacerbations) were assessed by logistic regression. Age, gender, age of asthma onset and tabaquism were analyzed as covariates. HDM sensitisation was also included in separate models.

Results: Prevalence of ascariasis was 4.1%, but sensitisation was high, 63.3%. HDM were frequent sensitisers (Bt, 66.7% and Dp, 59.8%). No association was found between infection and any outcome of asthma severity. Even after adjustment by HDM sensitisation, *Ascaris* sensitisation remained associated with severe dyspnea [aOR 2.58 95% CI 1.38–4.83; $P = 0.003$], >4 emergency room visits in the last year [aOR 2.15 (1.02–4.57), $P = 0.045$] and dyspnea with physical activity [aOR 2.27 (1.20–4.04) $P = 0.01$]. However, association with >12% change in FEV1 after bronchodilator use disappeared. When assessing sensitisation to the components Asc s1 (80%) and Asc 1 3 (22%), no significant relationships were found.

Conclusion: In this population of the tropics with low intensity ascariasis, our results support that *Ascaris* sensitisation increase the risk of asthma severity. Since most associations remained significant after adjustment by HDM sensitisation, it seems that *Ascaris* IgE response have independent effects on this disease. IgE serology to *Ascaris* 1, led to know that most patients were in fact exposed to this nematode, but it was probably insufficient to detect relationships between the complete antigenic extract and disease severity.

547 Survey of diagnosing asthma symptoms among medical students

Aliakhnovich, N¹

¹Clinical Immunology and Allergology, Vitebsk State Medical University, Vitebsk, Belarus

Background: Bronchial asthma (BA) affects approximately 300 million people worldwide (5%), making it one of the most common chronic diseases in the world. The actual rate of BA prevalence among the population of the country may vary from the official medical statistics. It's always been a problem to estimate the number of people suffering from the disease.

The aim is to survey the frequency and severity of asthma symptoms among students and identify those with high risk of BA development.

Method: We developed a simple, pre-interview screening questionnaire consisting of 12 questions. Every question has the own value for its importance as the pathognomonic criterion or the severity level of a symptom. Five hundred and twelve students were enrolled in the study with the help of the online variant of asthma questionnaire, placed on the educational site www.e-vsmu.by from 5/11/2012 to 12/11/2012. Participants were divided in five subgroups according to the total score gained. Twenty-two persons were excluded as they left some spaces blank.

Results: Four hundred and ninety persons include 386 female (79%) and 104 male students (21%), junior students (91%) showed the greatest activity.

Four hundred and thirty-three (88.6%) participants regarding the total score were included into the first subgroup (0–3 points), i.e. they had no signs of BA.

Three persons (0.6%) entered the fourth (19–23 points) and the fifth (24–30 points) subgroups, having the maximum score and therefore showing the obvious signs of the disease.

Fifty-three persons (10.8%) entered the second (26) and third (27) subgroups were likely to have BA, their answers were

analyzed thoroughly. The data obtained specified that 27 of them (51%) required additional allergic survey; 7 (13.2%) need the therapeutic consultation.

Conclusion: It is established that 5.5% of responders had high risk of asthma development or had signs of mild BA and required additional allergic inspection; 1.4% – therapeutic survey for the suspicion of nonspecific obstructive disease of lungs.

Regarding the level of risk group established asthma specialists should be aware of the great potential of questionnaires as a method of diagnosing asthma on its early stages

The data obtained from 300 participants are similar to those obtained from the further study of 500 participants. The results are believed to be relevant for larger groups of population and therefore the questionnaire developed can be used as an effective method of identifying asthma.

549 House dust mite sensitisation is the main risk factor for the increase in prevalence of wheeze in 13–14 year old schoolchildren in Guangzhou city, China

Li, J¹; Wang, H¹; Chen, Y²; Zheng, J¹; Wong, GW³; Zhong, N¹

¹Allergy and Clinical Immunology, The First Affiliated Hospital of Guangzhou Medical College, Guangzhou, China; ²Guangdong Pharmaceutical University, Guangzhou, China; ³Paediatrics, The Chinese University of Hong Kong, Hong Kong, China

Background: Little is known about time trend of prevalence of asthma and the association of its change with allergen sensitisation in schoolchildren in China. The purpose of this study was to determine the changes in prevalence of asthma and allergen sensitisation in schoolchildren in a period of over 15 years.

Method: A total of 6928 schoolchildren aged 13–14 years in 2009 were recruited for the study using the Phase III Protocol of the International Study of Asthma and Allergic disease in Childhood (ISAAC) and 2531 of them underwent skin prick test for seven common aeroallergens. The results were compared with those obtained in the Phase I (1994/95) and III (2001/02) ISAAC studies.

Results: The prevalence of asthma ever and current wheeze increased from 3.9% and 3.4% in 1994, to 4.6% and 4.8% in 2001 ($P < 0.001$), and to 6.9% and 6.1% in 2009 ($P \leq 0.008$). The prevalence of higher degree of skin response to house dust mites (HDMs) and cat, and atopic index increased significantly in all children in 2010 when compared with that in 2002 ($P < 0.001$). Prevalence of wheeze remained unchanged in subjects without sensitisation to any tested allergen includ-

ing HDMs ($P > 0.05$). Sensitisation to HDMs, especially *Dermatophagoides Pteronyssinus* (Der-p) was associated with increase in prevalence of wheeze.

Conclusion: The prevalence of wheeze and sensitisation to common aeroallergens in secondary schoolchildren in Guangzhou China has remarkably increased since 1994. Sensitisation to HDMs, Der-p in particular is an important risk factor for the increase in prevalence of wheeze in this group of population in Guangzhou city.

551 Gender difference of asthma and its comorbidities incidences – a 5-year population survey

Yeh, K-W¹; Yu, C-H²; Chan, P-C¹; Horng, J-T²; Huang, J-L¹

¹Division of Pediatric Allergy Asthma and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan; ²Department of Computer Science and Information Engineering, National Central University, Taoyuan, Taiwan

Background: The comorbidities of asthma are not uncommon, and they would make asthma hard to be controlled without concomitant management. The aim of this study was to investigate the incidence of asthma and its comorbid conditions of sleep apnea, gastroesophageal reflux (GER), obesity, sinusitis, bronchiectasis, chronic obstructive pulmonary disease (COPD), and vocal cord dysfunction (VCD) during 5 years period.

Method: This study was based on continuous data from the Taiwanese National Health Insurance Research Database which contained 98% of registry files of all 22.60 million populations. Asthma was selected with code 493.xx of the International Classification of Disease, 9th Revision, and Clinical Modification. Asthma case was defined as the first-rank diagnosis occurred more than three times within 6 months. A total 739 027 newly diagnosed asthma patients (388 596 in male, 350 431 in female) and 22 822, 663 (11 574 319 in male and 11 248 344 in female) non-asthma populations during 2003 to 2008 were analysed. Each disease was defined with corresponding ICD-9 code.

Results: Generally, asthma patients encountered more bronchiectasis (OR 4.28, $P < 0.001$), sleep apnea (OR 1.57, $P < 0.001$), GER (OR 1.57, $P < 0.001$) COPD (OR 4.01, $P < 0.001$), sinusitis (OR 2.11, $P < 0.001$) than non-asthma populations but not the case of obesity (OR 1.00, $P = 0.88$). However, VCD was less common in asthma patients (OR 0.81, $P < 0.001$). The most common comorbidity of asthma was COPD in both genders; and it was significant that male asthmatic patients had higher incidence of COPD

(12.7%) than females (7.19%, OD 1.88 $P < 0.001$). Although the incidence rates of sinusitis were close (6.44% in males and 6.24% in females), it was more common in male asthmatic patients. The incidence of bronchiectasis was 3.33% in female patients and 2.57% in males. Female patients also had 1.3 and 2-fold of risk to have VCD and obesity respectively than males. The incidence of sleep apnea were only 0.28% in males and 0.15% in females, but it was significantly common in male asthmatic patients (OR 1.93, $P < 0.001$).

Conclusion: Comorbid conditions were common in asthma patients. Those sleep apnea, sinusitis, and COPD were significant in male patients. Female patients presented more common in GER, Bronchiectasis, obesity and VCD.

552

Levels of serum 25-OH vitamin D in low income asthmatic children living in the Colombian Caribbean area

Garavito, G¹; Egea, G¹; Denny, R²; Visbal, L¹; Lecompte, N¹; Navarro, E¹; Bacerril, M³; Escamilla, JM⁴; Sánchez-Borges, M⁵; Egea, E¹; Grupo de Investigación en Inmunología y Biología Molecular

¹Universidad del Norte, Barranquilla, Colombia; ²Fundación Cardio Infantil, Bogotá, Colombia; ³Centro Médico La Raza, Mexico, Mexico; ⁴Universidad de Cartagena, Cartagena, Colombia; ⁵Universidad Central de Venezuela, Caracas, Venezuela

Background: Vitamin-D deficiency has been described as a marker of asthma severity in children. To date there are no studies searching level of VitD in asthmatic Colombian children and little is known about the relationship between vitamin-D and asthma severity in pediatric asthma living in poor and marginalized communities in Latin America. The purpose of this study was to determine the levels of vitamin-D and total IgE in asthmatic pediatric patients with asthma living in Barranquilla, a city of the Colombian Caribbean area and associate with the severity of the diseases.

Method: This was a case-control study. Samples from 64 Asthmatic children, and 127 healthy controls, aged between 5 and 11 years, were enrolled. Clinical variables paraclinical results were recorded in a modified questionnaire from ISAAC-2009. The clinical diagnosis was made according to the parameters described by GINA-2009. Serum vitamin-D and total IgE concentrations were determined by ELISA. The association between vitamin-D levels, total IgE-levels, socioeconomic status, clinical onset and severity of asthma was made for differences in variance.

Results: The mean serum levels of vitamin-D for the group of patients was 66.12 ng/ml. For the control group was

68.50 ng/ml. Only two patients and controls had insufficient vitamin D levels (<30 ng/ml). None of the subjects was VitD deficient. Total IgE in patients was elevated in 26 of 64 (40.1%), 36/127 controls had elevated total IgE (28.3%). No significant differences were found between VitD levels in patients and controls (ANOVA $P = 0.220$). Nor between total IgE levels and VitD (ANOVA $P = 0.087$). Comparing VitD against the socioeconomic, clinical onset and severity of asthma, we found no differences in these variables (ANOVA $P = 0.8244$, $P = 0.993$ and $P = 0.265$, respectively).

Conclusion: These preliminary results seems to show that in asthmatic children living in marginal and poor areas in the Colombian Caribbean area have not hypovitaminosis-D. There was also no association between vitamin-D levels and the severity of asthma.

553

Modelling and predicting trends in childhood asthma hospital readmission over time

Vicendese, DA¹; Olenko, A²; Dharmage, SC³; Allen, KJ⁴; Tang, ML⁵; Abramson, MJ⁶; Erbas, B¹

¹School of Public Health, La Trobe University, Bundoora, Vic., Australia; ²Department of Mathematics and Statistics, La Trobe University, Bundoora, Vic., Australia; ³School of Population Health, The University of Melbourne, Carlton, Vic., Australia; ⁴Department of Allergy & Immunology, Royal Children's Hospital, Parkville, Vic., Australia; ⁵Department of Epidemiology & Preventive Medicine, Monash University, Melbourne, Vic., Australia

Background: Little is known about childhood asthma re-admissions over time. Seasonality is an important marker of total environmental load or triggers such as high pollen and pollution exposure and respiratory virus infections. Being aware of the days/times of the year when childhood asthma readmissions are expected to increase may assist in the achievement of better and more efficient health service planning. Our objective was to model and predict daily trends of childhood asthma readmissions over time in Victoria, Australia and ascertain whether these trends varied by age and gender.

Method: We used a large database of 75 000 childhood asthma admissions from the Department of Health Victoria, Australia between 1997 and 2009. Daily counts of asthma hospital readmissions over time were modelled using a semi parametric Generalised Additive Model (GAM) method and by sex and age group. We also predicted daily readmissions using these models.

Results: $N = 2401$ asthma readmissions within 28 days occurred between fiscal

years of 1997 and 2009. Of these, $n = 1358$ (57%) were boys. Boys displayed greater variability between season and age group, $P = 0.001$. Autumn, tended to have a greater risk of readmission for two groups: 6–12 year girls – borderline significant, OR = 1.51, 95% CI 0.98–2.33, $P = 0.06$; and 13–18 year boys OR 1.61, 95% CI 1.01–2.59, $P = 0.047$. Spring showed a disproportionate risk only for 6–12 year boys OR = 1.59, 95% CI 1.08–2.36, $P = 0.02$. Readmissions during the grass pollen season in Victoria were associated with all age groups in boys, $P = 0.01$. The smooth function of time was also significant, $P < 0.0005$, and indicated that, after initial declining readmission counts to about 2001, they subsequently began to increase returning to roughly initial levels at study end. Predictions suggested readmissions would continue to increase by up to 5% on average overall, with boys in the 2–5 year age group experiencing the largest increase.

Conclusion: The strong seasonal component in readmissions suggest that: clinical services may need to revise procedures and be alert to possible greater risk of readmission at certain times of the year, especially for younger age groups; health services management may need to adjust resource allocation and planning.

554

Nasopharyngeal bacterial colonization in asthmatic children and healthy controls

Roumpedaki, E¹; Xepapadaki, P¹; Doudoulakis, A²; Lebesse, E³; Passioti, M¹; Skevaki, C¹; Taprantzi, P³; Tsakanikos, M⁴; Tsolia, M⁵; Dutre, T⁵; Bachert, C⁵; Lindholm, L⁵; Lukkarinen, H^{7,8}; Jartti, T⁸; Albert, F⁹; Graser, A¹⁰; Geißdörfer, W⁹; Bogdan, C⁹; Zimmermann, T¹¹; Finotto, S¹⁰; Sobańska, A¹²; Kowalski, ML¹²; Papadopoulos, NG¹

¹2nd Pediatric Clinic, Allergy Department, National and Kapodistrian University, Goudi, Athens, Greece;

²Department of Microbiology, P & A Kyriakou Children's Hospital, Athens, Greece; ³2nd Pediatric Clinic, National and Kapodistrian University, Goudi, Athens, Greece; ⁴ENT Department, P & A Kyriakou Children's Hospital, Athens, Greece; ⁵Upper Airway Research Laboratory (UGENT), Ghent University Hospital, Ghent, Belgium; ⁶Department of Medical Microbiology and Immunology, University of Turku, Turku, Finland; ⁷Turku University Hospital, The Research Centre of Applied and Preventive Cardiovascular Medicine (CAPC), Turku, Finland; ⁸Department of Pediatrics, University of Turku, Turku, Finland; ⁹Friedrich-Alexander Universität Erlangen-Nürnberg, Microbiology Institute – Clinical Microbiology, Immunology and Hygiene, Erlangen, Germany; ¹⁰Friedrich-Alexander Universität Erlangen-Nürnberg, Molecular Pneumology Institute Universitätsklinikum, Erlangen, Germany; ¹¹The Children's Hospital in Erlangen, Allergy and Pneumology Department, Erlangen, Germany; ¹²Department of Immunology, Rheumatology and Allergy, Faculty of Medicine, Central University Hospital, Medical University, Lodz, Poland

Background: PreDicta (Post-Infectious Immune Reprogramming and its association with Persistence and Chronicity of

Respiratory Allergic diseases), is an EU FP7 funded project investigating the influence of viral and bacterial respiratory infections on the persistence of asthma and their correlation with underlying immune responses. Part of this project involves a 2 year multi-centre childhood cohort study across major cultural and climatic European regions, namely Athens (Greece), Ghent (Belgium), Turku (Finland), Erlangen (Germany) and Lodz (Poland). The aim of the present study is to compare nasopharyngeal bacterial colonization between asthmatic children and healthy controls.

Method: To date, 131 cases with mild-moderate persistent asthma (as per GINA) aged 4–6 years old and 35 healthy controls, matched per age, defined as children having no history of asthma, wheeze and/or atopic illness have been recruited. As part of the baseline assessment, a nasopharyngeal swab was obtained (ESwab 482CE, Copan, Italia), in the absence of an apparent infection. Standard microbiology procedures for the identification of *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Staphylococcus aureus* and *Streptococcus pyogenes* (Group A strep) followed thereafter. Statistical analysis was performed with the Graph-Pad Prism5 software.

Results: One or more of five culprit bacteria was isolated in 62% of cases and 75% of controls. In 43.5% of asthmatic children and 58.3% of controls only one of the five indicator bacteria studied was identified, in 14.5% of asthmatic children and 16.7% of controls two of these facultative respiratory pathogens were isolated, whereas in 4% of cases and none from the healthy controls 3 or more bacteria were isolated. 37, 9% of cases and 25% of controls were negative for all 5 culprit bacteria. *Haemophilus influenzae* was identified in 23.7% of asthmatic children and 31.4% of controls, *Staphylococcus aureus* in 26.7% of asthmatic children and 17.1% of controls, *Moraxella catarrhalis* in 18.3% of asthmatic children and 20% of controls, *Streptococcus pyogenes* in 0.8% of cases and 5.7% of healthy control, while *Streptococcus pneumoniae* was isolated from 14.5% of asthmatic children and 11.4% of healthy controls. Differences between case and control children were not statistically significant.

Conclusion: In this population, bacterial colonization at baseline is as frequent in asthmatic children as in healthy controls.

555

Risk factors for asthma in children with history of respiratory syncytial virus and rhino virus infections

Kartasasmita, CB¹; Saptaputra, W²; Mutyara, K²; Setiabudhiawan, B¹

¹Child Health, Universitas Padjadjaran, Bandung, Indonesia; ²Research Unit, Universitas Padjadjaran, Bandung, Indonesia

Background: Many respiratory viruses can provoke acute asthma symptoms. Respiratory syncytial virus (RSV) and rhino virus (RV) infections are associated with asthma development. Recently, studies showed that early RV infections are the important predictors for developing asthma in high risk children. The aim of this study is to determine the risk factors of developing asthma in children with history of RSV, RV and RSV+RV infections.

Method: Interview on the health condition, demography, history of atopy and asthma in children and family, were obtained, using structured questionnaires. A skin prick testing (SPT) were performed according to the method of the Australasian Society of Clinical Immunology and Allergy Inc (ASCI). The allergens were obtained from Statallergenes, Antony, France. The skin prick testing was performed to 12 common standardised allergens: Blomia-tropicalis, Dermatophagoides farinae, Dermatophagoides pteronyssinus, Alternaria alternata, Aspergillus mix, Cockroach, Egg white, Egg yolk, Chocolate, Cat dander, Shrimp and Soya. Histamine was used as a positive control and saline as negative control. Skin prick test were read 15 min after placement and are considered positive when the induration diameter ≥ 3 mm than the negative control of at least one allergen.

Results: A total 86 children with history of viral respiratory infections (35 RSV, 25 RV, 16 RSV+RV), and 246 control were enrolled. Atopy was found in 60% RSV, 92% RV, 43.8% RSV+RV, and 75.2% control respectfully. History of atopy among children with RSV is 34.3%, RV 28%, RSV+RV 18.8% and control 28.9%. Asthma, based on 'history of wheezing in the last 12 months' was found in 17.1% RSV, 8% RV, 6.3% RSV+RV, and 4.5% control group respectfully. The number of asthma was significantly higher in the RSV group compared to control group ($P = 0.01$), but not in the RV ($P = 0.341$) and RSV+RV groups ($P = 0.538$). The risk factors were low mother education ($P = 0.01$), no home ventilation ($P = 0.04$), smoking in the living room, and no ventilation ($P = 0.02$).

Conclusion: More atopy is found in RV group, asthma is high in RSV group. The risk factors for asthma are low mother

education, no home ventilation, smoking in the living room, and no ventilation.

557

Trends in asthma: dispensing of medications, hospitalisations and mortality from 1998 to 2011 in a Brazilian city

Mello, LM¹; Silva, AS¹; Martinez, EZ¹; Barros, CGC¹; Ferreira, JBB¹; Vassimon, C²; Nunes, AA¹

¹Department of Social Medicine, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil; ²Municipality Health Department, Ribeirão Preto, Brazil

Background: Several studies have linked the poor control of asthma to lack of proper treatment and situations that hinder the uninterrupted access to the medication are among the reasons of this inappropriate control. Our purpose was to assess the availability and consumption of medications for asthma, the hospitalisations and mortality for asthma between December 1998 and December 2011, at the Public Health System of a city in the Southeastern of Brazil.

Method: A time series description of the annual consumption of the aminophylline (tablets), salbutamol sulfate (tablets and oral solution), salbutamol (albuterol) spray and beclomethasone dipropionate spray was conducted from the records of the Health Information System of the Health Department of the city. The data about hospitalisations and mortality for asthma were obtained from the DATASUS records (Ministry of Health, Brazil).

Results: Aminophylline (3100 tablets consumed in 1998; 768 199 tablets consumed in 2007 and 90 714 tablets in 2011) and salbutamol sulfate (1159 tablets consumed in 1998; 257 022 tablets consumed in 2007 and 90 714 tablets consumed in 2011; 102 vials consumed in 1998; 16 440 vials consumed in 2004 and 6476 vials consumed in 2011) have been available for treating asthma in our country for several years but their dispensing/consumption showed a clear decrease. The salbutamol spray and beclomethasone dipropionate spray was made available to the users of the public health system from 2004 what possibly explains the growing consumption of this date (58 and 201 units consumed in 2005 to 13 310 and 9194 respectively in 2011). The number of hospitalisations for asthma presented a decrease of 88% in this period: from 682 admissions in 1998 to 81 admissions in 2012. Data suggests that asthma mortality remained variable but it outlined a downward trend. It was observed 9 deaths in 1996, 5 in 1997, 7 in 1998, 8 in 1999, 12 in 2000, 6 in 2001, 6 in 2002, 10 in 2003, 3 in 2004, 5 in 2005,

10 in 2006, 2 in 2007, 2 in 2008, 5 in 2009 and 7 in 2010.

Conclusion: The points of change in the time series description of the dispensing/consumption of the medications for asthma and the decreasing trends of the indicators on hospitalisations and mortality for asthma probably reflect, among other factors, the public policies implemented in past years that aim to ensure the provision of first line medications and pharmaceutical care on asthma as well as the improvement of the clinical practice on asthma.

558

Patterns of adherence to asthma medication among asthmatics with acute exacerbations following up in a tertiary allergy institution in Kuwait

Al-Ahmad, M¹; Arifhodzic, N²; Radha, P¹

¹Allergy Department, Al-Rashed Allergy Center, Kuwait, Kuwait; ²Al-Rashed Allergy Center, Kuwait, Kuwait

Background: Asthma Exacerbation remains a leading cause of asthma morbidity.

It is usually an indication of poor asthma control, frequently related to non-adherence to medical therapy.

Method: Adherence to the prescribed medication was assessed in 1244 randomly selected adult asthmatics with acute exacerbation, being followed up in a tertiary allergy institute. Patients age 15 years and above, both sex, with a physician diagnosis of allergic or non-allergic asthma, on established regular anti-inflammatory treatment to maintain good asthma control, were included. Compliance to the medication in different age groups (15–40, 41–60, >60 years old), and among different asthma severity grades, based on clinical score, ER visits, and FEV1 values in the previous year from patient's records, were compared.

Results: Allergic asthma was the most common type of asthma phenotype in our group of patients (72.9% vs 27.1%; $P < 0.0001$). There was no gender differ-

ence (70.3% male vs 75.1% female; $P < 0.06$). No difference has been found in the type of sensitisation to inhalant allergens between citizens and expatriates (73.7% vs 71.2%; $P < 0.35$). Majority of the patients had mild or moderate asthma (34.6% & 41.2%), while only 3.9% had severe form. Adherence to asthma medication was in general very poor, but was significantly more present in young age group when compared with elderly (33.1% vs 47.1%; $P < 0.001$). Adherence was better in citizens than in expatriates (42.9% vs 30.6%; $P < 0.001$). With regard to asthma severity, patients with mild as well as severe form of asthma, had the poorest compliance (34.6% & 55.1%).

Conclusion: Non-adherence to asthma medication is an important factor for poor asthma control, not only in mild, but also in more severe cases. Permanent education to increase awareness is the cornerstone of better asthma control.

Poster Session 10

What do the new murine models tell us?

559

Inhibitory effects of staphylococcal enterotoxin type A (SEA) and B (SEB) on mice bone marrow eosinophil adhesion *in vitro*

Ferreira-Duarte, AP¹; Squebola-Cola, D²; Mello, G²; Antunes, E²; DeSouza, I¹

¹Biology and Physiology, Faculty of Medicine of Jundiaí, Jundiaí, Brazil; ²Faculty of Medical Sciences – State University of Campinas (UNICAMP), Pharmacology, Campinas, Brazil

Background: Clinical evidences have shown a strong correlation between SEA and SEB on bronchial asthma exacerbation. We have described that SEA and SEB mice airways exposition aggravate the pulmonary allergic inflammation by exacerbation of lung eosinophils (EO) infiltration and increased bone marrow (BM) eosinopoiesis. In the present study we evaluated the influence of mice pulmonary SEA or SEB exposition on BM EO *in vitro* adhesion induced by eotaxin. Effect of the incubation of BM EO from naïve animals with SEA and SEB on *in vitro* adhesion induced by eotaxin and RANTES was also evaluated.

Method: BALB/C mice femurs were removed after killing, flushing with 2.5 ml of Iscove's medium and submitted to granulocyte isolation protocol. The supernatants were collected, centrifuged (500 g for 10 min at 4°C), and the cell pellets resuspended to 4×10^6 cells/ml. Adhesion assays were carried out in 96-well plates pre-coated with recombinant mouse VCAM-1 and ICAM-1 (2.5 µg/ml) for 30 min in the presence of eotaxin or RANTES. The EO adhesion was calculated by measuring EO peroxidase activity on adherent cells.

Results: Mice airways exposition to SEA for 48 h reduced the adhesive response of BM EO in ICAM-1 coated plates (eotaxin/untreated mice BM EO: 12.7 ± 2.5 ; eotaxin/SEA treated mice BM EO: 8.0 ± 0.1 OD/EOS $\times 10^6$ cells). Similar results were observed with BM EO from SEB-treated mice for 16 h (eotaxin/untreated mice BM EO: 15.7 ± 2.7 ; eotaxin/SEB treated mice BM EO: 9.5 ± 1.2 OD/EOS $\times 10^6$ cells). BM EO from naïve mice incubated *in vitro* with SEA or SEB for 2 h also exhibited reduced adhesive response when stimulated by eotaxin or RANTES (eotaxin/VCAM-1: 20.5 ± 2.5 ; SEA: 12.3 ± 1.0 ; SEB:

13.2 ± 1.2 ; RANTES/VCAM-1: 10.7 ± 0.8 ; SEA: 2.6 ± 0.8 ; SEB: 5.2 ± 0.6 ; eotaxin/ICAM-1: 14.0 ± 1.3 ; SEA: 10.2 ± 0.9 ; SEB: 10.7 ± 1.5 ; RANTES/ICAM-1: 11.9 ± 1.1 ; SEA: 6.6 ± 0.5 ; SEB: 6.1 ± 1.0 OD/EOS $\times 10^6$ cells).

Conclusion: The inhibitory effect of SEA and SEB on BM EO *in vitro* adhesion suggests a role of these toxins on downregulation of BM EO surface adhesion molecules which contribute to clarify the mechanisms involved on the association between *Staphylococcus aureus* infections and allergic respiratory disease.

Financial support: Fundação de Amparo a Pesquisa do Estado de São Paulo (2009/16522-0; 2012/05561-8).

560

SEC14L3 is de-regulated in murine experimental asthma via miRNA-mediated down-regulation of CREB

Bartel, S¹; Schulz, N¹; Theis, F²; Alessandrini, F³; Take-naka, S¹; Eickelberg, O¹; Krauss-Etschmann, S¹

¹Helmholtz Zentrum München, Comprehensive Pneumology Center, University of Munich, Member of the German Center for Lung Research, München, Germany; ²Department of Genome Oriented Bioinformatics, Helmholtz Zentrum München, München, Germany; ³Helmholtz Zentrum München, ZAUM-Center for Allergy and Environment, München, Germany

Background: MicroRNAs are small non-coding RNAs essential for immune function and lung development. Previously, we found increased pulmonary expression of miR-17, -144, -21 & -205 in ova-induced asthma, which correlated with decreased mRNA & protein levels of putative target genes CREB1 and its co-activators CRTC1 & 3. Here, we hypothesised that these miRNAs regulate CREB/CRTC-mediated gene transcription and sought to elucidate this interdependency *in vitro* and identify new CREB-dependent target genes relevant for asthma.

Methods: To substantiate the miRNA-mediated regulation of CREB and CRTCs, miRNAs were overexpressed (precursor miRNAs) or inhibited (antimiRs) in murine (MLE-12) or human bronchial epithelial cells (16-HBE). Analysis was performed by RT-qPCR & Western blotting. Putative CREB1 targets relevant for asthma were selected if decreased in lungs

of mice with ova-induced asthma and by presence of CRE elements in their promoter region. Periodic acid schiff (PAS) stain and SEC14L3 immunostaining was performed.

Results: The mRNA and protein level of CREB & CRTCs were mediated by *in vitro* overexpression and silencing of the respective miRNAs. Among the 35 candidate CREB1 targets, we chose SEC14L3, a transporter for hydrophobic ligands, for further analysis. Its mRNA expression could be modulated *in vitro* by miRNA silencing in a CREB1-dependent manner. Immunostainings of lung sections showed specific SEC14L3 expression in ciliated airway epithelial cells, which was decreased in asthmatic mice. This coincided with goblet cell metaplasia and loss of epithelial integrity.

Conclusion: Using miRNA profiles as pre-selection tools, we identified SEC14L3 as new target that might be useful for monitoring the integrity of the respiratory epithelium in experimental asthma.

561

A novel nDer p2-conjugated TLR7 down-modulates Th2-associated lung inflammation by using a therapeutical protocol

Pratesi, S¹; Nencini, F¹; Petroni, G¹; Cardilicchia, E¹; Fili, L¹; Casini, A¹; Guarna, A¹; Romagnani, S¹; Parronchi, P¹; Maggi, E¹; Vultaggio, A¹

¹University of Florence, Florence, Italy

Background: Modified adenine (MA) binding TLR7 represent a new group of adjuvants for immunotherapy. We investigated the *in vitro* and *in vivo* effects of a synthetic MA [4-(6-amino-9-benzil-8-idrossi-9H-purin-2-ilsulfanil) butirrico 2,5-diossopirrolidin-1-il estere] called SA26E, chemically conjugated to purified allergen (nDer p2). In particular we analyzed the modulation of Th2-mediated immune response in a murine model of lung inflammation through a therapeutical protocol.

Methods: C57Bl/6 mice were intraperitoneal (i.p.) immunised with nDer p2 at d0 and d7. At d14 mice were intratracheally challenged with nDer p2, while at d21 and d28 mice were i.p. treated with an administration of nDer p2-conjugate or nDer p2

alone as control. At d49 and d53 mice were again challenged with nDer p2 and then sacrificed at d56 for the analysis.

Results: SA26E stably conjugated to allergen, maintained the ability to trigger HEK293 cell line transfected with murine TLR7. Moreover, it downregulated eosinophils in bronchoalveolar lavage of mice treated with nDer p2-SA26E conjugated but not with nDer p2 alone. This effect was associated with a shift in the antibody profile from a type 2- (IgE and IgG1) to a type 1-associated (IgG2a) humoral response. A reduction of IL-13 and a parallel increase of IFN- γ was observed in the supernatants of lung mononuclear cells *in vitro* stimulated with antigen. A concomitant significant increase of IL-10 levels was also found in the supernatants of nDer p2-stimulated cells of spleen and draining lymph nodes.

Conclusion: These data suggest that the use of nDer p2-SA26E conjugated regulates and redirects the immune response in a therapeutic fashion and may constitute an useful approach for immunotherapy of allergic diseases.

562

A novel mouse model for house dust mite driven allergic asthma

Canbaz, D¹; Logiantara, A¹; van Ree, R¹; van Rijt, LS¹
¹Academic Medical Center, Experimental Immunology, Amsterdam, The Netherlands

Background: House dust mite (HDM) allergens are a major cause of asthma worldwide, by provoking an aberrant immune response. Allergic asthma is a Th2 cell mediated disease. The airway epithelium is considered to be essential for sensitisation to HDM. HDM can trigger Toll like receptor 4 on epithelial cells and thereby activate dendritic cells (DCs). These activated DCs migrate to lung draining lymph nodes to induce the polarisation of Th2 cells in allergic asthma. We hypothesised that mice could be sensitised by intratracheal instillation of *in vitro* HDM pulsed DCs without the mucosal exposure to HDM.

Method: DCs were cultured from bone marrow and were pulsed overnight with 100 μ g/ml HDM. DCs were thoroughly washed to remove residual HDM. Cytokine production and phenotype of the DCs were detected by ELISA and FACS, respectively. Next, HDM-DCs or unpulsed DCs were administered intratracheally in Balb/c mice to induce sensitisation to HDM. After 10 days, mice were challenged with HDM intranasally. Two days later, airway hyperreactivity (AHR) to metacholine was measured. One day later, eosino-

phil recruitment to the bronchoalveolar space, Th2 cytokine production by lung draining lymph nodes, HDM specific IgE/IgG1 ratio in serum and airway histology by PAS staining in lung slides were analyzed.

Results: DCs pulsed with HDM showed a significant increase in IL-6 and IL-10 cytokine production and upregulation of CD80 and CD86 co-stimulatory factors compared with unpulsed DCs. We observed a trend for an enhanced AHR to metacholine in mice sensitised with HDM pulsed DCs compared with control mice. In concordance, eosinophil levels in the bronchoalveolar space and Th2 cell cytokines like IL-4, IL-5 and IL-13 were increased. Airway histology showed a significant increase in eosinophilic inflammatory infiltrates and mucus production. We did not observe a significant difference in the serum HDM specific IgE and IgG1 compared with the control.

Conclusion: These results suggest that HDM pulsed DCs are sufficient to induce an eosinophilic airway inflammation in response to HDM. This model can be used to investigate how dendritic cells control the HDM driven immune response.

563

Influence of VEGF and TNF antagonism on zonula occludens proteins in an experimental asthma model

Yuksel, H¹; Yilmaz, O¹; Karaman, M²; Firinci, F³; Turkeli, A¹; Toprak Kanik, E¹; Inan, S⁴

¹Pediatric Allergy and Pulmonology, Celal Bayar University, Manisa, Turkey; ²Multidisciplinary Laboratory, Dokuz Eylul University Faculty of Medicine, Izmir, Turkey; ³Pediatric Allergy and Immunology, Dokuz Eylul University, Izmir, Turkey; ⁴Histology and Embryology, Celal Bayar University, Manisa, Turkey

Background: Epithelial barrier dysfunction is important in asthma and allergic response pathogenesis. Therefore, it is a new therapeutic target candidate. We aimed to investigate influence of dexamethasone as classical therapeutic agent, anti-TNF (etanercept) that is used in difficult asthma and anti-VEGF (bevacizumab) that is an angiogenesis inhibitor, on zonula occludens proteins in experimental asthma model.

Methods: We developed experimental asthma model using intraperitoneal (IP) and inhaled ovalbumin in 32 BALB/c mice investigated in four groups. Each group received either IP saline, IP etanercept, IP bevacizumab or IP dexamethasone. Occludin, claudin and junctional adhesion molecule (JAM) were stained in left middle lobe samples immunohistochemically with indirect avidin-peroxidase method and semi-quantified with H-score.

Results: We observed a significant difference of occluding, claudin and JAM H-score between the four groups ($P < 0.001$). Median H-scores were 93, 177, 280 and 198 for occludin; 82, 193.5, 274 and 202.5 for claudin; 130, 210, 288 and 210 for JAM in groups 1 to 4 respectively. Comparisons in groups of 2 showed that, all three zonula occludens protein H-scores are significantly lower in saline group compared to each treatment group. However, H-scores of the zonula occludens proteins were not significantly different between etanercept and dexamethasone groups. Moreover, bevacizumab group had higher H-scores for all proteins when compared to dexamethasone group but only higher JAM H-score when compared etanercept group.

Conclusion: Antagonism of VEGF restores epithelial barrier to a higher extent when compared to both dexamethasone and etanercept. This result may be promising for development of new therapeutic agents.

564

Effect of CpGODN on the level of interleukin-17 in ovalbumin allergic asthma model of mice

Li, M¹; Wu, C¹; Zhang, H¹; Li, C¹

¹The Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical College, Wenzhou, China

Background: Establishing mice acute asthma model, interfered in CpGODN and observing the variation of IL-17 levels in blood serum and BALF, and investigate the possible mechanisms of CpGODN's immunoregulatory.

Method: The asthmatic models were established by ovalbumin (OVA) sensitised and challenged to murine. 40 male Balb/c mice were randomly divided into 4 groups, including control group (group A), Asthma model group (group B), Dexamethasone control group (group C), CpGODN treatment group (group D). Group B were sensitised by OVA and establishing the acute mice asthma model. Group A were sensitised and challenged by Saline instead of OVA, group C and D sensitised both by OVA and to be administered with DXM (0.5 mg/kg) and with CpGODN (50 μ g each one) by i.p. before challenging a day and a hour respectively. After challenged for 24 h in all groups, BALF and blood serum were collected and the total cell numbers in BALF were counted. Lung were fixed and sections were used for histopathological examination, the level of IL-17 in BALF and blood serum were measured by Enzyme-linked immunosorbent (ELISA).

Results: (1) The condition of inflammatory cell in BALF and pulmonary histopathol-

ogy: the total cell numbers, the absolute numbers of EOS, the ratio of EOS to the total cell numbers (EOS%) in group B were markedly higher than those of group A ($P < 0.01$ respectively); those of group C and D compared with group B show significant decrease ($P < 0.01$ respectively). There were little infiltration of inflammatory cells in bronchial lumen, mucus secretion decreased significantly, and no obvious thickening of bronchial smooth muscle in Group C and D compared with group B.

(2) The level of IL-17 in BALF and blood serum in group B (57.50 ± 5.59 pg/ml, 255.08 ± 49.46 pg/ml), were significantly higher than those in group A (53.02 ± 3.31 pg/ml, 217.52 ± 36.30 pg/ml) ($P < 0.05$); group C and group D (52.62 ± 4.11 pg/ml, 218.35 ± 44.08 pg/ml; 51.37 ± 3.24 pg/ml, 207.20 ± 20.08 pg/ml) were significantly decreased compared with B ($P < 0.01$, $P < 0.01$).

Conclusion: The level of IL-17 in BALF and serum were significantly increased in the mice acute asthma model sensitised by OVA, by administered with CpGODN it were significantly decreased and airway inflammation were improved, similar to Dexamethasone decreased the level of IL-17 in the BALF and serum and improved airway inflammation.

565

Effects of arginase inhibition and inhaled L-arginine administration on airway histology in a murine model of acute asthma

Arkan Ayyıldız, Z¹; Karaman, M²; Firinci, F¹; Kiray, M³; Bağrıyanık, A⁴; Yılmaz, O²; Uzuner, N¹; Karaman, O¹

¹Department of Pediatrics, Division of Pediatric Allergy, Dokuz Eylül University Faculty of Medicine, Izmir, Turkey; ²Department of Experimental Animal Laboratory, Dokuz Eylül University Faculty of Medicine, Izmir, Turkey; ³Department of Physiology, Dokuz Eylül University Faculty of Medicine, Izmir, Turkey; ⁴Department of Histology and Embryology, Dokuz Eylül University Faculty of Medicine, Izmir, Turkey

Background: Increased arginase activity in the airways decreases L-arginine and cause deficiency of bronchodilating and anti-inflammatory NO. It is suggested that arginine and arginase inhibitors may have therapeutic potential in the treatment of asthma. We aimed to investigate the effects of inhaled L-arginine and arginase inhibitor N ω -hydroxy-nor-L-arginine (nor-NOHA) on airway histology of acute asthma using a murine model.

Method: Forty-two BALB/c mice were divided into six groups; I, II, III, IV, V (placebo) and VI (control). All groups except the control were sensitised and challenged with ovalbumin. After methacholine

induced bronchoconstriction, the mice were given inhaled L-arginine (Group I), nor-NOHA (Group II), L-arginine-nor-NOHA combination (Group III), budesonide (Group IV) and placebo, respectively. SaO₂ was measured by pulse oximeter just before (0 min) and 5 min after 25 mg/ml concentration of methacholine. A third measurement of SaO₂ was also obtained 15 min after drug administration in these five study groups. Inflammation in the lung tissues of the sacrificed animals was scored to determine the effects of the study drugs (score 0; none, 1; mild, 2; moderate, 3; severe).

Results: Inflammatory scores significantly improved in groups receiving study drugs when compared with placebo and they were similar when compared with budesonide. SaO₂ had a tendency to increase after L-arginine administration and this increase was significant ($P = 0.043$).

Conclusion: In our study we demonstrated that L-arginine and arginase inhibition improved inflammatory scores and L-arginine improved SaO₂ in acute model of asthma. Combination treatment had no additive effect on either of these therapies.

566

Murine asthma model has enhanced response to ozone but not in sham model

Uh, S-T¹; Kim, YK¹; Kim, KU¹; Park, SW²; Jang, AS²; Kim, DJ²; Park, CS²

¹Soon Chun Hyang Univ. Seoul Hosp., Seoul, Korea; ²Soon Chun Hyang Univ. Bucheon Hosp., Bucheon, Korea

Background: Ozone is well known as an important component of ambient air pollutants. Ozone can aggravate respiratory symptoms in patients with bronchial asthma, but, not in healthy persons. We hypothesised asthma itself may show different response to ozone compared to non-asthma. This study was performed to evaluate the differences of response to ozone between normal mice and murine asthma model in terms of oxygen toxicity.

Method: Three PPM of ozone was exposed to OVA-albumin-induced murine asthma model (OVA-model) for 3 h at 3, 7, 14, 21 days after completion of asthma model. Airway responsiveness to methacholine was measured after completion of asthma model. Bronchoalveolar lavage, protein extraction from lung for Western blot and immunohistochemistry (IHC) of 4-HNE, PCNA, Nrf-2 were performed at before and each ozone exposure day.

Results: Airway responsiveness to methacholine had significantly higher methacholine-induced Penh in OVA-model than that

in sham group ($P < 0.05$). The number and percentages of eosinophil increased at baseline, 3 and 7 days and returned to nearly baseline at 14, 21 days after completion of OVA-model. In sham group, the expression of 4-HNE increased at only 14 days, but, in OVA-model, the expression of 4-HNE more increased at baseline compared to sham group, and ozone more increased 4-HNE expression at 3 days and 7 days. In IHC of 4-HNE, staining majority cells were bronchial epithelium, the intensity were similar with the results of Western blot. In both OVA-model and sham group, PCNA expression was not different from baseline to 21 days. But the expression of PCNA was significantly increased in OVA-model compared to those in sham group. PCNA was significantly expressed in bronchial epithelium in OVA-model during experimental period. Nrf-2 was not expressed in sham model, but, in OVA-model, Nrf-2 expressed at baseline, 3 and 7 days.

Conclusion: Asthma itself is vulnerable to oxygen toxicity in spite of activation of anti-oxidant system.

567

Unmethylated CpG-ODN administration attenuates cockroach extract-induced pulmonary inflammation in a dose dependant manner

Sohn, J-H^{1,2,3}; Lee, JH^{2,4}; Choi, J-M^{3,5}; Park, J-W^{2,4}

¹Department of Life Science, Research Institute for Natural Sciences, Hanyang University, Seoul, Korea; ²Department of Internal Medicine and Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea; ³Hanyang Biomedical Research Institute, Seoul, Korea; ⁴Department of Internal Medicine, Division of Allergy and Immunology, Yonsei Medical School, Seoul, Korea; ⁵Department of Life Science, Research Institute for Natural Sciences, Hanyang University, Seoul, Korea

Background: Unmethylated CpG can induce Th1 and regulatory T cell immune responses and may modulate ovalbumin-induced mouse allergic asthma. However, the effects of CpG in a cockroach allergen asthma model have not yet been elucidated.

Objective: To evaluate whether unmethylated CpG can prevent the development of indoor allergen-induced allergic asthma, and whether dose variation of unmethylated CpG can make difference in response.

Methods: Effects of CpG were determined using a cockroach allergen-induced mouse model. To make allergic asthma model, *Blattella germanica* (CR) allergens were administered six times over 3 weeks. For low dose model, CpG (3 μ g) was only once treated at the last CR administration. For high dose CpG model, CpG were co-administered six times with CR.

Methacholine airway hyperresponsiveness (MCh-AHR), inflammatory cell quantification and cytokine level in BAL fluid, and lung histology were evaluated. Lung homogenates were analysed using FACSARIA.

Result: Co-administration of CpG with CR allergen prevented the development of MCh-AHR and allergic inflammation in a CR-induced asthma mouse model. Eosinophils, lymphocytes, macrophages and neutrophils in BAL fluid were decreased by the co-administration of unmethylated CpG. In low dose CpG model, enhanced Th1 response and reduced Th2 response were demonstrated by increased level of IFN- γ and decreased level of IL-5. However, all of the levels of IL-5, IL-13, and IFN- γ were declined in high dose CpG model. The expression of IL-10, IL-12p70, IL-17 in BAL fluid and lung homogenate was also affected by the CpG. Peribronchial, perivascular inflammation, and goblet cell hyperplasia in respiratory epithelium were also markedly attenuated by the CpG.

Th1, Th2 and Th17 responses were all declined by high dose CpG treatment. On the other hand, low dose CpG treatment appeared to have therapeutic effect increasing Th1 response and attenuating Th2 and Th17 responses.

Conclusion: These findings suggest that unmethylated CpG may have role in the immune modulation of allergic asthma induced by indoor allergens.

568

Sensitisation by subcutaneous route is superior to intraperitoneal route in the induction of asthma by house dust mite in a murine model

Aun, MV¹; Saraiva-Romanholo, BM²; Arantes-Costa, FM²; Almeida, FM²; Martins, MA²; Kalil, J¹; Giavina-Bianchi, P¹

¹Division of Clinical Immunology and Allergy, University of Sao Paulo School of Medicine, Sao Paulo, Brazil; ²Internal Medicine, University of Sao Paulo School of Medicine, Sao Paulo, Brazil

Background: Experimental animal models have been the foundation of basic research in asthma, aiming the better understanding of its pathogenesis and the creation of new strategies of treatment. We present a new murine model of asthma by mite, comparing two different routes of sensitisation.

Method: The protocol lasted 30 days. BALB/c mice were divided into six groups, which were sensitised subcutaneously (s.c.) or intraperitoneally (i.p.), with saline (negative control) or *Dermatophagoides pteronyssinus* (Der p), 50 or 500 mcg, in three injections. Subsequently mice underwent intranasal challenge with Der p or saline for 7 days and were sacrificed 24 h after

the last challenge. We measured specific IgE anti-Der p, eosinophilic density in peribronchovascular space and airway remodeling.

Results: Both animals sensitised i.p. and s.c. produced specific IgE, with no difference between them. Peribronchovascular eosinophilia increased only in mice receiving lower doses of Der p. However, only the group sensitised with 50 mcg of Der p through s.c. route showed significant airway remodeling.

Conclusion: In this murine model of asthma, both pathways of sensitisation led to the production of specific IgE and eosinophilia in the airways. However, only the s.c. route was able to induce remodeling. Furthermore, lower doses of Der p used in sensitisation were better than higher ones, suggesting immune tolerance. More studies are needed to evaluate the development of bronchial hyperresponsiveness in this model, but it can already be replicated in experiments to create new therapeutic drugs or immunotherapeutic strategies.

569

The role of endoplasmic reticulum stress in the pathogenesis of bronchial asthma

Lee, YC¹; Kim, SR¹; Kim, DI¹; Lee, KS¹; Park, SY¹; Jeong, JS¹

¹Department of Internal Medicine, Chonbuk National University Medical School, Jeonju, Korea

Background: Despite of a plenty of studies on endoplasmic reticulum (ER) stress in various inflammatory diseases, there is scarce information on ER stress in bronchial asthma. In this study, we aimed to elucidate the role of ER stress in the pathogenesis of bronchial asthma.

Method: Using mice sensitised with ovalbumin (OVA) and lipopolysaccharide (LPS) and then challenged with OVA (OVA_{LPS}-OVA mice) as well as OVA-sensitised and-challenged mice (OVA-OVA mice), we have investigated whether ER stress is involved in the pathogenesis of bronchial asthma. Moreover, we also determined the level of an ER stress marker in blood from patients with asthma.

Results: The OVA_{LPS}-OVA mice showed that the expression of ER stress markers and the protein levels of unfolded-protein response (UPR)-related markers in lung tissues were significantly increased after OVA challenge. Moreover, we found that ER stress markers in peripheral blood mononuclear cells from human asthmatics were dramatically increased compared with those from healthy controls. In OVA_{LPS}-OVA mice, 4-phenylbutyric acid (4-PBA), a chemical chaperone significantly reduced the increases in ER stress, inflammatory

cytokines, dendritic cells (DCs) infiltration with Toll-like receptor 4 (TLR4) expression, airway inflammation, and bronchial hyperresponsiveness, while it further enhanced the increase of IL-10. Additionally, the established asthmatic features of OVA-OVA mice were substantially attenuated by 4-PBA administered after completion of OVA challenge.

Conclusion: These results indicate that ER stress may be implicated in the pathogenesis of bronchial asthma, at least in part, through modulation of immune responses.

570

Goblet cell metaplasia and mucus production are airway-region specifically regulated in murine allergic asthma

Vock, C¹; Yildirim, AO²; Wagner, C³; Wegmann, M⁴; Fehrenbach, H¹

¹Member of the German Center for Lung Research, Division of Experimental Pneumology, Airway Research Centre North, Research Center Borstel, Borstel, Germany; ²Helmholtz Zentrum Munich, Member of the German Center of Lung Research, Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Neuherberg, Germany; ³Member of the German Center for Lung Research, Division of Invertebrate Models, Airway Research Centre North, Research Center Borstel, Borstel, Germany; ⁴Member of the German Center for Lung Research, Division of Asthma Mouse Models, Airway Research Centre North, Research Center Borstel, Borstel, Germany

Background: Goblet cell metaplasia (GC) and mucus hyperproduction are major hallmarks of allergic bronchial asthma, the most common chronic inflammatory disease worldwide.

Exaggerated mucus production is a critical factor impairing lung function of asthmatic patients. It might result in plugging of small airways with detrimental impact on ventilation of the connected alveoli.

In acute and chronic mouse models of allergic asthma, GC and mucus hyperproduction are seen in proximal but not distal airways although expression of interleukin (IL)-13, the main trigger of mucus hyperproduction, was significantly elevated along the entire airway tree. Effects of IL-13 are mediated by the IL13 receptor (IL-13R) composed of the IL-4R α and IL-13R α 1 subunit and the down-stream signalling cascade. We therefore hypothesised that differences in the IL-13/IL-13R signalling cascade are responsible for airway-region specific mucus production.

Methods: BALB/c mice were sensitised with OVA/Alum and subsequently challenged with OVA for one (acute) or twelve weeks (chronic). Systematic uniform samples obtained from lungs fixed with 4% paraformaldehyde were embedded into paraffin, sectioned and stained with periodic acid Schiff (PAS) to analyse mucus production. Lungs were inflated with

RNAlater® to microdissect proximal and distal airways for qRT-PCR. Laser-based microdissection (LCM) of airway epithelial cells was performed on cryofixed lungs.

Results: QRT-PCR analyses of microdissected airways and of LCM-derived airway epithelial cells revealed significantly lower abundance of IL-13R α 1 in distal airways, whereas IL-4R α mRNA levels were similar between proximal and distal airways. Investigations of major transcription factors implicated in GC and mucus production down-stream the IL-13/IL-13R signalling, displayed a significant airway-region specific gene expression pattern: i) expression levels of Spdef and FoxA3, both known to promote GC and mucus production, were barely expressed in distal airways but significantly up-regulated in proximal airways of OVA-treated mice; ii) repressors of GC and mucus production, namely FoxA2 and Nkx2-1, were both higher expressed in distal airways of PBS mice and repressed in proximal and distal airways of OVA-treated mice.

Conclusion: We suggest that the attenuation of the IL-13 dependent signalling cascade in distal airways protects from mucus plugging and thus, prevents impaired ventilation. Elucidating such protective mechanisms may help identifying new targets for asthma therapy.

571

Antigen-specific regulation of asthmatic responses in mice by intratracheal exposure to anti-ovalbumin monoclonal antibody Fab fragments

Yoshino, S¹; Mizutani, N¹; Ammori, Y¹; Torii, H¹

¹Pharmacology, Kobe Pharmaceutical University, Kobe, Japan

Background: Fab fragments (Fabs) maintain the ability to bind to specific antigens but lack effector functions due to the absence of the Fc portion. In the present study, we investigated whether Fabs of an antigen-specific monoclonal antibody (mAb) were able to regulate asthmatic responses in mice.

Method: Asthmatic responses were induced in BALB/c mice sensitised with ovalbumin (OVA) on days 0 and 14 followed by intratracheal (i.t.) challenge with OVA on days 28, 29, 30, and 35. Fabs prepared by the digestion of an anti-OVA IgG1 (O1-10) mAb with papain were i.t. administered 30 min before the last antigenic challenge on day 35. Normal IgG Fabs were used as a control.

Results: I.t. administration of O1-10 but not control Fabs markedly suppressed the early and late phases of asthmatic responses when the *in vivo* responses were

evaluated by specific airway resistance (sRaw). The significantly reduced number of neutrophils in bronchoalveolar lavage fluids (BALF) was seen in mice treated with O1-10 Fabs. The lung tissue of mice administered with O1-10 Fabs also had decreased infiltration of neutrophils as well as less production of IL-1 β . The suppression of asthmatic responses by O1-10 Fabs was associated with significantly lower levels of mMCP-1 in sera as well as complement C3a in BALF. Similar results were obtained when Fabs of anti-OVA IgG2b mAb (O2B-3) were i.t. administered. In contrast, neither systemic injection of O1-10 Fabs nor i.t. administration of intact O1-10 affected the asthmatic responses. *In vitro* studies revealed that the capture of OVA by O1-10 Fabs resulted in the prevention of the subsequent binding of intact anti-OVA antibodies to the captured OVA when small but not large amounts of the intact antibodies were present.

Conclusion: Asthmatic responses appear to be downregulated by the i.t. exposure to Fabs of an antigen-specific mAb via a mechanism involving the capture of antigen by Fabs in the respiratory tract before the interaction of intact antibody and antigen that is essential for induction of asthmatic responses.

572

The TLR3 ligand poly(I:C) promotes the production of IL-17 by NKT cells

Nencini, F¹; Vultaggio, A¹; Pratesi, S¹; Petroni, G¹;

Romagnani, S¹; Maggi, E¹

¹University of Florence, Florence, Italy

Background: Allergic asthma is associated with a Th2-oriented response, even if recently Th17 cells have been shown to play a role in the pathogenesis of asthma, favouring the recruitment of neutrophils and the evolution to chronicity. The airway-targeted viruses consistently exacerbate bronchial asthma and the early rhinovirus infection, rather than other respiratory viruses, have been clearly associated with wheezing and pediatric asthma.

In this study, we examined the activity of the dsRNA poly(I:C) and the modified adenine SA-2 (ligands of TLR-3 and TLR-7, respectively) on the IL-17 production by α -GalCer-activated iNKT cells, a model mimicking the activity of both RNA viruses and microbial antigens on inflamed lung tissue.

Methods: The *in vitro* effect of poly(I:C) on the release of IL-17A by spleen and lung CD1d-activated iNKT cells with α -galactosylceramide (α -GalCer) has been initially evaluated.

The effect of TLR-3L was also investigated in an α -GalCer-induced murine models of lung inflammation. The inhibition of IL-17A by Toll-like receptor (TLR) 7 agonists in the same *in vitro* and *in vivo* models has been lastly analysed.

Results: Poly(I:C) upregulated the *in vitro* IL-17A production by CD1d-driven invariant NKT cells, without modifying type 1 and type 2 cytokines. The two stimuli selectively upregulated IL-17A serum levels *in vivo*. Their i.t. administration resulted in increased airway hyper-reactivity (AHR), neutrophilia in bronchoalveolar lavage and airway inflammation, which were inhibited by anti-IL-17A antibody. Poly(I:C) effects were attributable to IL1 β and IL-23 release from dendritic cells, as showed by inhibition with neutralising antibodies. TLR7 agonists inhibited the IL-17A production by poly(I:C) plus α -GalCer in the same models. Such effect was associated with the increased production by DC of IL-17A-inhibiting cytokines (IL-27 and IL-10) and the dampening of IL-1 β and IL-23.

Conclusion: Allergic asthma is worsened by viral infections sustained by rhinoviruses, which express TLR3 sequences, while are less related to other respiratory viruses (influenza, respiratory syncytial), that exhibited both TLR3 and TLR7 sequences. These data suggest that IL-17A induced by TLR3L is a relevant signal also for inducing acute lung inflammation.

573

TLR-3 triggered aggravation of experimental asthma depends on IL-17

Lunding, LP¹; Webering, S¹; Vock, C¹; Hölscher, C²; Fehrenbach, H¹; Wegmann, M¹

¹Research Center Borstel, Airway Research Centre North, Member of the German Center for Lung Research, Priority Area Asthma & Allergy, Borstel, Germany; ²Research Center Borstel, Priority Area Infections, Borstel, Germany

Background: Exacerbations are a common event in asthma patients that cause considerable morbidity on patients. The inflammatory phenotype of these exacerbations in sputum as well as broncho-alveolar lavage (BAL) is characterised by a heterogeneous infiltrate with eosinophils and large numbers of neutrophils. In epidemiological studies viral infections of the respiratory tract were identified as the main trigger. During their replication all major respiratory viruses produce double stranded RNA (dsRNA) as an intermediate. This can be sensed by the immune system via toll-like receptor 3 (TLR3). Therefore, we propose a key role for TLR3 in the process underlying acute allergic asthma exacerbations. Thus, it was the aim of our study i) to establish a mouse model

of TLR3-triggered asthma exacerbation and ii) to investigate the responsible mechanisms.

Method and Results: Local activation of TLR3 by intra-nasal (i.n.) application of synthetic ligand poly(I:C) induced an exacerbation of experimental allergic asthma in mice characterised by increased release of pro-inflammatory cytokines, enhanced mucus production, and pronounced airway hyperresponsiveness (AHR). Additionally, considerable infiltration of neutrophils and T helper 17 (TH17) cells was observed. However, poly(I:C) failed to induce an exacerbation in mice lacking IL-17A, which indicates a central role for this cytokine in mediating TLR3 triggered asthma exacerbation. Furthermore, in IL-23p19 mice, which do not have any IL-17 producing $\gamma\delta$ T cells and TH17 cells, local application of poly(I:C) again provoked asthma exacerbation.

Conclusion: Therefore, we suggest IL-17 producing iNKT cells as main cell type mediating the TLR3 triggered exacerbation of allergic asthma in mice.

574

The effectiveness of *Nigella sativa* in preventing the airway remodeling through an immune response modulation in the asthmatic mouse model

Barlianto, W¹; Chandrakusuma, H²; Widodo, A³; Karyono, S³

¹Division Allergy-Immunology Department of Pediatric, Faculty of Medicine University of Brawijaya, Malang, Indonesia; ²Division Respiratory, Department of Pediatric, Faculty of Medicine University of Brawijaya, Malang, Indonesia; ³Department of Pharmacology and Therapy, Faculty of Medicine University of Brawijaya, Malang, Indonesia

Background: Chronic inflammation on asthma is dominated by T CD4⁺ lymphocytes which secrete cytokines T helper-2 (Th2) type, playing a role in airway remodeling. Apart from a Th2 cell role, T CD4⁺CD25⁺FoxP3⁺ lymphocytes, a T regulator, plays a vital role in asthma. *Nigella sativa* is potential as immunomodulator, having an antiinflammatory effect. This research aims to prove the efficacy of *Nigella sativa* in preventing the airway remodeling in the asthmatic mouse model.

Method: This research used female mice aged 8–10 weeks. The experimented animal was divided into 3 randomised groups, group I was sensitised and challenged with ovalbumin and served as a positive control, group II was neither sensitised nor challenged and served as a negative control, group III were sensitised and challenged with ovalbumin and treated with *Nigella sativa* (dosage 4.8 g/kg body weight/day). Mice were sensitised to ovalbumin intra-

peritoneally and were challenged via nasal inhalation with ovalbumin 1% for 6 weeks to induce airway remodeling. *Nigella sativa* was administered orally everyday and started as the same time as sensitisation. Twenty four hours after the final challenge, mice were sacrificed and the lung was taken for analysis. The left lung was examined for histopathological purpose while another was for flowcytometry examination. Flowcytometry analysis was to measure the lymphocyte count of T CD4⁺ and T CD4⁺CD25⁺FoxP3⁺. Bronchoalveolar lavage fluid was collected to measure TGF- β level.

Results: *Nigella sativa* administration decreased the lymphocyte count of T CD4⁺ and raised the lymphocyte count of T CD4⁺CD25⁺FoxP3⁺. The suppression of T CD4⁺ lymphocytes was consistent with apoptosis increase of T CD4⁺ lymphocytes after *Nigella sativa* administration. The increase of T CD4⁺CD25⁺FoxP3⁺ lymphocytes was correlated to the increase of TGF- β level. The increased level of TGF- β after *Nigella sativa* administration was influential in inducing T CD4⁺ into T CD4⁺CD25⁺FoxP3⁺ lymphocytes. Histopathological examination showed *Nigella sativa* administration prevent the increased epithel thickness, smooth muscle thickness, subepithelial fibrosis thickness, and the numbers of goblet cell.

Conclusion: *Nigella sativa* can prevent the occurrence of airway remodeling in the asthmatic mouse model via an immune response modulation.

576

A first sensitisation to food allergen induces an aggravation of asthmatic phenotype in a house dust mite asthmatic model

Lair, D^{1,2,3}; Roussey-Bihouée, T^{1,3,4}; Rolland-Debord, C^{1,2}; Aubert, P^{3,5}; Gourbeyre, P⁶; Cheminant, M-A^{1,2,3}; Sagan, C⁷; Neunlist, M^{3,5}; Bodinier, M⁶; Magnan, A^{1,3,8}

¹INSERM, UMR 1087/CNRS 6291, Nantes, France; ²Université de Nantes, IRS-UN, l'Institut du Thorax, Nantes, France; ³Département Hospitalo-Universitaire DHU2020, Nantes, France; ⁴Département de Pédiatrie, CHU Nantes, Nantes, France; ⁵INSERM U913, Nantes, France; ⁶INRA, Unité BIA1268, Nantes, France; ⁷CHU Nantes, Service d'Anatomie et Cytologique Pathologiques, Nantes, France; ⁸CHU Nantes, l'Institut du Thorax, Service de Pneumologie, Plate-Forme Transversale d'Allergologie, Nantes, France

Background: The atopic march represents the passage of atopic dermatitis in infants, often with food allergy, to allergic asthma in childhood or young adulthood. The objective of this work is to develop a murine model of successive food and respiratory allergy, in order to mimic the atopic march and to investigate whether pre-existing food allergy worsens the asthmatic phenotype.

Method: Balb c mice are first sensitised 3 times intraperitoneally then challenged 3 times intra gastrically to ovalbumin (OVA) as food allergen. Thereafter, to induce asthmatic phenotype, mice are sensitised 4 times transcutaneously and challenged 2 times intra nasally by an extract of *Derma-tophaeoides farinae*. Digestive analyses consist in measurements of permeability *in vivo* and *ex vivo* in Ussing chamber with measurement of total transit time. Respiratory function is assessed with whole body plethysmography. White blood cells, lung cellularity and bronchoalveolar lavage (BAL) composition are analysed by flow cytometry. Local or systemic response is analyzed by the cytokine secretion in BAL or spleen. Total and specific IgE serum levels are assessed by ELISA.

Results: In mice with food sensitisation and respiratory allergy compared to mono-allergic mice, we found a significant worsening of the asthmatic phenotype with

- (1) increased airway hyperresponsiveness,
- (2) pulmonary and LBA hypercellularity and
- (3) a hypersecretion of proinflammatory cytokines in the lung and BAL.

Moreover, this aggravation is also reflected systemically with hypersecretion of total and specific IgE. In the other hand the digestive bi-allergic mice exhibit a tendency to increased intestinal permeability, without changing the total transit time.

Conclusion: In our new model, the asthmatic phenotype is exacerbated by pre-existing food allergy, assuming an increased susceptibility in response to environmental allergens. Now, this new characterised model will be used in order to understand the mechanisms which allow the passage of food allergy to respiratory allergy, and testing new therapies to prevent this evolution.

577

Beneficial effects of arginase inhibitor and inhaled L-arginine administration on airway histology in a murine model of chronic asthma

Arikan Ayyıldız, Z¹; Karaman, M²; Tuncel, T³; Kiray, M⁴; Bağrıyanık, A⁵; Yılmaz, O²; Uzuner, N⁵; Karaman, O³

¹Dokuz Eylül University Faculty of Medicine, Izmir, Turkey; ²Dokuz Eylül University Faculty of Medicine, Department of Experimental Animal Laboratory, Izmir, Turkey; ³Department of Pediatrics, Division of Pediatric Allergy, Dokuz Eylül University Faculty of Medicine, Izmir, Turkey; ⁴Department of Physiology, Dokuz Eylül University Faculty of Medicine, Izmir, Turkey; ⁵Department of Histology and Embryology, Dokuz Eylül University Faculty of Medicine, Izmir, Turkey

Background: Increased arginase activity in the airways induces reduced bioavailability of L-arginine and cause deficiency of bron-

chodilating and antiinflammatory NO. These also enhance formation of peroxynitrite and L-ornithine, which are all involved in pathogenesis of asthma. Therefore, arginine and arginase inhibitors may have therapeutic potential in the treatment of acute and chronic asthma. Using a murine model of chronic asthma, we investigated the effects of inhaled L-arginine and arginase inhibitor N ω -hydroxy-nor-L-arginine (nor-NOHA) on airway histology of asthmatic lung tissue.

Method: Forty-two BALB/c mice were divided into six groups; I (control), II (placebo), III, IV, V and VI. Mice except control group were sensitised by an intraperitoneal injection of ovalbumin with alum adjuvant and then challenged with an aerosol of ovalbumin on 3 days of the week for 8 weeks beginning from the 21st day of the study. Lung histology was evaluated after 7 days of treatment period with inhaled L-arginine (Group III), inhaled nor-NOHA (Group IV), L-arginine-nor-NOHA combination (Group V), budesonide (Group VI) and placebo. Interleukin-4, IL-5 and IL-13 levels were studied in lung homogenates with ELISA.

Results: Group I was similar with budesonide group in lowering all histologic parameters. Results of groups treated with nor-NOHA (Group IV and V) were also similar with budesonide group except epithelial thickness. Decrease was only noted in IL-4 in the group receiving nor-NOHA.

Conclusion: In our study we demonstrated that inhaled L-arginine administration alleviated all histologic parameters similar to budesonide and treatment with arginase inhibitor improved not all but some of the pathologic changes in chronic asthma. Combination therapy had no additive effect on either treatments.

578

The effect of anti-IL-33 antibody and soluble ST2 in a murine model of allergic asthma

Kang, HS¹; Lee, HY¹; Rhee, CK¹; Lee, SY¹; Moon, HS¹; Kwon, SS¹

¹Internal Medicine, The Catholic University of Korea, Seoul, Korea

Background: Interleukin (IL)-33 is involved in development of allergic inflam-

mation, and soluble ST2 (sST2) is a fusion protein that neutralises IL-33 activity. Therefore, we aimed to investigate whether Anti-IL-33 and sST2 reduced inflammation in asthma.

Method: Forty BALB/c mice were used. In group A (control group, $n = 6$), mice were sensitised and challenged with saline. Group B [ovalbumin (OVA) group, $n = 6$] mice received subcutaneous and intranasal OVA challenge. In group C (control goat IgG group, $n = 6$), mice were injected intraperitoneally with control IgG before OVA challenge. In group D (anti-IL-33 group, $n = 8$), anti-IL-33 was injected intraperitoneally before challenge. In group E (control human IgG group, $n = 6$), mice were injected intraperitoneally with control IgG before OVA challenge. In group F (sST2 group, $n = 8$), sST2 was injected intraperitoneally before challenge. We evaluated the number of eosinophils, neutrophils, lymphocytes and macrophages in bronchoalveolar lavage (BAL) fluid; airway hyperresponsiveness (AHR) to methacholine; and IL-4, IL-5, IL-10 and IL-13 in BAL fluid.

Results: Anti-IL-33 treatment significantly reduced the number of eosinophils in BAL fluid. IL-4, IL-5, IL-10, and IL-13 in BAL fluid were also significantly decreased after Anti-IL-33 treatment. sST2 also significantly reduced the number of eosinophils in BAL fluid. IL-4, IL-5, IL-10, and IL-13 in BAL fluid were also significantly decreased after sST2 treatment. Airway hyperresponsiveness to methacholine was decreased when treated with both Anti-IL-33 antibody and sST2.

Conclusion: Anti-IL-33 antibody and sST2 has a therapeutic potential for allergic asthma.

579

The effect of influenza virus infection on airway inflammation in a murine model of asthma

Kim, H-H¹; Chun, YH²; Yoon, J²; Lee, JS²

¹Pediatric Allergy, Bucheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Bucheon-si Kyunggi-do, Korea; ²Pediatrics, The Catholic University of Korea, Seoul, Korea

Background: Respiratory viral infection is a leading cause for asthma exacerbation. It

is known that respiratory syncytial virus (RSV) infection induces IL-5 dependent eosinophilia and Th2-polarised immunity, which are typical phenotypes of virus-induced aggravation in allergic asthma. However, although influenza infection, accounts for a relatively high proportion of severe cases, pathogenic mechanism of asthma exacerbation is not well understood. The purpose of this study was to examine the mechanism of inflammatory cells accumulation into the airways after influenza infection in a murine model of asthma by comparing typical cytokines related to the production of eosinophils and neutrophils.

Method: House dust mite (HDM)-sensitised BALB/c mice were used as a model of influenza-asthma exacerbations. The airway cholinergic hyperresponsiveness, pulmonary histopathological changes, and bronchioalveolar lavage fluid (BALF) analysis including cells and cytokine influx were observed 24 h following intranasal infection with influenza viruses.

Results: Following influenza infection in asthmatic mice, the airway responsiveness to inhaled methacholine was increased ($P < 0.05$). Lung tissue showed enhanced infiltration of neutrophils rather than eosinophils. BALF analysis revealed a statistically significant increase in neutrophils and decrease in eosinophils in the influenza-infected mice as compared to the HDM-sensitised mice. These changes were associated with an increased level of neutrophil chemotactic factor (CXCL1) in the BALF ($P < 0.05$). However, the levels of IL-1 β -another important cytokine released in response to increased neutrophils- showed no changes. Regarding to eosinophils, eotaxin levels found in the BALF showed a significant reduction ($P < 0.05$). However IL-5, and regulated and normal T cell expressed and secreted (RANTES) did not show significant changes.

Conclusion: Our results show that the mechanism of asthma exacerbations by influenza infection may result from neutrophilic inflammation rather than eosinophilic inflammation. Cytokines may play a role in neutrophilic inflammation.

Poster Session 11

Improved assessment of asthma

580

Pre-olympic examination of six Indonesian athletes in 2012: the introduction of a short version allergy questionnaire for athletes

Pramadita, D¹; Kurniawan, AA²; Rahadian, B¹; Munasir, Z^{3,4}

¹Technology, and Sport Health, Center for Science, The Ministry of Youth and Sport Affair, Jakarta, Indonesia;

²Indonesia Sports Medicine Center, Jakarta, Indonesia;

³Department of Child Health, Faculty of Medicine University of Indonesia, Jakarta, Indonesia;

⁴Department of Child Health, Ciptomangunkusumo Hospital, Jakarta, Indonesia

Background: Elite athletes especially Olympic athletes have bigger prevalence of allergy than general population up to 22% in Olympic Winter Games. There has never been a thorough examination of allergy in Indonesian Olympic athletes. In year 2012 Indonesian medical team for Olympic started to use questionnaire that has been validated in Indonesian language using only 13 questions that could be scored to screen allergic diseases in Indonesian athletes.

Method: From total 22 Indonesian athletes went to Olympic 2012, only 6 athletes has been thoroughly fulfill all examination (blood test, urine test, Hepatitis B Antigen and Antibody test, functional movement screening test, ECG, spirometry) including allergy questionnaire. The original questionnaire consisted of 25 questions and in this examination we only use 13 questions which have scores.

Results: There were 2 of 6 athletes scored positive for the questionnaire (score 5 and 7). Both of them answer yes for question related to rhinitis allergy (Do you frequently sneeze, have a running, itchy nose (apart from colds)?) that scored 5 which is also minimum score for positive allergy. And one of them had mild obstructive abnormality according to spirometry result with FEV₁ was 69.13% and FEV₁/FVC was 65.68%. This athlete is a weightlifter and he had history of smoking in the past.

Conclusion: Although some studies showed that pulmonary function test in athletes might have higher standard than general populations, we found one athlete with mild obstructive abnormality whom weightlifter and had history of smoking. Unfortunately this finding was found in very small number of athlete underwent complete medical examination. We do not

have enough published data about pulmonary function test in Indonesian Olympic athletes nor allergy examinations. In reality enforcement of healthy habit such as quit smoking is still a problem in Indonesian athletes other than allergic diseases. Allergic diseases were less found than injuries and infections in athletes thus yet to be priority in Indonesian athlete medical examination. Recommendation of better and holistic medical and physical examination of all Indonesian elite athlete especially Olympic athletes is needed, considering the management system of sports medicine is yet to be achieved well at this moment.

581

Challenge with inhaled mannitol, 17 case reports of chronic cough and dyspnea with exercise

Burgos Montero, AM¹; Gonzalez Sanchez, LA¹; Candon Morillo, R¹; Ruiz Leon, B¹; Moreno Mata, E¹

¹Allergy Department, Hospital La Mancha Centro, Alcazar de San Juan, Spain

Background: The aim of this study is to report 17 patients with respiratory disease (chronic cough or dyspnea during exercise) with previously normal spirometry, and the outcome after inhaled mannitol challenge.

Method: We made challenge with mannitol in 17 patients that come to Allergy Department to study and evaluate cough causes and their treatment. They had pathology of coughing during rest or during exercise, with normal spirometry and negative bronchodilator previously test for the realisation of provocation. They were prepared by removal of drugs that could interfere in the test and were given an informed consent (in case of teenagers, parents were given it). Nevertheless, all challenges were completed despite the cough that occurs during the test.

Results: We made 17 challenges in our patients. Among these, 10 patients come for exercise-induced bronchospasm and 7 come for chronic cough with over 6 months of evolution. Seventy were women and 30% were men. Over 41.1% were adolescents and the rest, 58.8%, were adults. Only two of them had gastric pathology (11.7%) and six of them had history of atopy (35.2%). One of patients

was smoker and two of them were ex-smokers. As a result, we had 3 positive challenges, two of them were teenagers and the other was an adult, but all with the diagnosis of exercise-induced bronchospasm and none of them had gastric pathology. None of the patients with a diagnosis of chronic cough was positive.

Conclusion: Bronchial challenge with mannitol can be used in daily practice, but it is not yet a substitutive of other bronchial challenge tests. Although we have few cases in this report, we observed that patients who were positive in the test match in pathology and almost in age (adolescents with exercise-induced bronchospasm) and chronic cough patients have a negative test, despite coughing during the challenge. Maybe, we need more patients and more studies to make conclusions, but in this report we can see that these data suggest us that the mannitol test is not very specific in the diagnosis of chronic cough, but it can be more useful to exercise-induced bronchoesasm.

582

Lifestyle factors as main causative factors apart from allergen sensitisation in the development of persistent nature of asthma from an intermittent one

Purushotam, D¹; Ahmed, M²

¹Mandore Satt. Hospital, Allergy, Jodhpur, India;

²Paediatric and Allergy, Masha Medical Center, Hyderabad, India

Background: We all know asthma as a chronic inflammatory condition of allergic etiology, so we tried to know factors responsible for development of persistent asthma in atopic patients who previously were intermittently symptomatic.

Method: We took 25 patients of persistent asthma as, of recent origin (criteria:- need of regular preventive inhalational medications on regular basis since last 1–5 years) and analysed their lifestyle of previous 5 years on 6 below mentioned points:

- 1 Change to sedentary life style.
- 2 Weight gain.
- 3 Any form of mental stress.
- 4 Change in dietary factors.

We took patients coming to our secondary ref feral hospital for continuous need

of preventive inhalers, we took patients from middle age i.e. from 30 to 35 years of age only of both sex.

Results: Out of 25 patients results for various factors were as follow:

- 1 Sedentary life style:-out of 25 patients, 20 developed need for regular medication use after their routine changed in such a way that most part of their job needed sedentary lifestyle and most of them do not pursue any regular exercise pattern.
- 2 Most of them gain weight in excess after which they needed preventers on regular basis.
- 3 Interestingly many of them started using preventers on regular basis after a severe emotional stress of medium duration, as determined by use of hypnotics or antidepressant medication. In our study we found that depression was a major risk factor for development of persistent symptoms in already intermittently symptomatic patients.
- 4 Interestingly majority of patients started needing preventers after they switched their diet from home based low fat diet to hotel based high fat diet, from regular frequent small meals to irregular large meals.

Conclusion: In conclusion we found that apart from allergic sensitisation lifestyle factors are key causative factors in transformation from intermittent to persistent symptomatology in allergic-asthmatic patients, establishing that persistent asthma is a chronic inflammatory disorder like diabetes and ischemic heart disease in genetically predisposed individuals needing multidisciplinary measures to prevent and treat persistent asthma apart from allergen avoidance and pharmacotherapy.

583

Comparison of two asthma control test for children: Childhood Asthma Control Test and Japanese Pediatric Asthma Control program

Sato, K¹; Oda, Y¹; Suzuki, S¹; Nezu, Y¹; Matsura, T¹; Watanabe, H¹; Nishimuta, T¹

¹National Shimoshizu Hospital, Pediatrics, Chiba, Japan

Background: Recent worldwide asthma treatment guidelines and consensus reports presented disease control as a goal of treatment. Control tests were recommended to assess asthma control simply and objectively. Childhood Asthma Control Test (C-ACT) was available for 4 to 11 aged children with asthma among many countries also in Japan. Japanese Pediatric Asthma Control program (JPAC) was developed in Japan locally, and had used as commonly

as C-ACT in Japan. We established differences between two control tests and relationship with health related quality of life to take advantage of them.

Method: Children with asthma aged 4–11 and their care givers who visited National Shimoshizu Hospital were recruited. Children in asthma exacerbation were excluded. Adequate children and care givers met doctor after they completed C-ACT and Japanese Pediatric Asthma Quality of Life Questionnaire 2001(JAQLQ). The doctors blinded to results of C-ACT and JAQLQ completed JPAC with interviewing about asthma control. Correlation between C-ACT and JPAC score, differences in answers of equivalent questions, and relationship with QOL were analyzed statistically. SPSS software ver. Seventeen were used for all statistical analysis.

Results: One hundred and ninety sets of two hundred three were obtained. Mean age of children was 7.4 years old. JPAC score was correlated with C-ACT score ($r^2 = 0.614$, $P < 0.01$). Concordance rate whether controlled or not among two control tests was 88.1%. Discordance between two control tests were observed mainly in case of not controlled. Differences between child's answer and caregiver's were largest in question about exercise induced bronchospasm or cough (discordance rate 29.8%). JPAC score was not well correlated with JAQLQ score ($r^2 = 0.282$).

Conclusion: C-ACT and JPAC were in another style but equivalent in outcome measures. Knowledge about differences between two tests and proper use is needed to assess childhood asthma adequately.

584

Spirometry and eNO values in healthy pre-school children from Navarra

Olaguibel, JM¹; Alvarez Puebla, MJ¹; Vela Vizcaino, C¹; de Esteban, B¹; Uribe San Martin, P¹; Cambra Contin, K²

¹SNS, Alergologia, Pamplona, Spain; ²Fundacion Miguel Servet, Pamplona, Spain

Background: Pre-school children can produce reproducible spirometric maneuvers, provided a well trained technician and appropriate incentive software are available. Its use in clinical practice has, however, been limited by lack of consensus in acceptability criteria and reference data. There was a lack of normal values for this age range in our area, so a survey was conducted to obtain spirometric reference values in a healthy pre-school children population from Navarra along with exhaled nitric oxide (eNO) values.

Method: Seventy five normal pre-school children were included and of these 60 were able to produce acceptable and repro-

ducible maneuvers, according to quality control criteria recommended by Aurora et al. (1).

Results: The regression model of natural lung function parameters and height had the best correlation. After accounting for height in the model, other physical traits did not contribute significantly. FVC, FEV1 and FEV0.5 all increase with height. Correlation coefficients were 0.88, 0.87 and 0.86 respectively. Reference equations were developed: $FEVC = -2.4365 + 0.0347 \cdot \text{height}$, $FEV1 = -2.0678 + 0.0297 \cdot \text{height}$, $FEV0.5 = -1.5383 + 0.0225 \cdot \text{height}$. There was a decrease in the ratio FEV1/FVC with increasing height. eNO values (Niox Minor/6 s) were obtained in 56 children. Mean value was 10 ppb (SD 5.2), values ranging from <5 to 18 ppb.

Conclusion: High quality spirometry can be obtained in the majority of pre-school children. Prediction equation for spirometry for pre-school children of our area, and normal eNO values are presented.

1. Aurora P, Stocks J, Oliver C, et al. Quality control for spirometry in pre-school children with and without lung disease. *Am J Respir Crit Care Med* 2004;169:1152–9.

585

Features of school-age children with asthma according to aeroallergen sensitisation and risk factors for uncontrolled asthma

Yavuz, ST¹; Kartal, O²; Arga, M¹; Güleç, M²; Gök, F³; Şener, O²; Sekerel, BE⁴

¹Department of Pediatric Allergy and Immunology, Gülhane Military School of Medicine, Ankara, Turkey;

²Department of Adult Allergy and Immunology, Gülhane Military School of Medicine, Ankara, Turkey;

³Department of Pediatrics, Gülhane Military School of Medicine, Ankara, Turkey; ⁴Pediatric Allergy and Asthma Unit, Hacettepe University School of Medicine, Ankara, Turkey

Background: Clinical phenotypes may vary among children with asthma according to the aeroallergen sensitisation status. The aim of this study is to document the demographic and laboratory features of school-age children with asthma and to determine factors associated with uncontrolled asthma.

Method: Children with physician-diagnosed asthma who attended to an outpatient pediatric allergy and asthma center were enrolled in the study. A questionnaire including demographic features and parameters to determine socioeconomic status were applied. Asthma control status of patients was evaluated according to GINA criteria. Laboratory investigations including complete blood counts with differential, total IgE levels, skin prick tests and pulmonary function tests were performed.

Results: A total of 159 children (96 male (60.4%); with a median age [interquartile range] of 8.0 [6.1–11.3] years were included. 45.9% of children had aeroallergen sensitisation. The age of onset of asthma symptoms were later (2.8 years [0.8–5.2] vs 5.5[2.0–7.2]), $P = 0.004$, family history of allergic disease was more frequent (52.1% vs 27.9%), total IgE levels (52.6 kU/l [28.8–113.9] vs 20.1[4.0–84.8]), $P < 0.001$ and eosinophil counts (250 [180–445] vs 145[110–243]), $P = 0.001$ were higher in children with aeroallergen sensitisation. Asthma was controlled in 50 children (31.4%), whereas partially controlled and uncontrolled in 55 (34.6%) and 54 (34.0%) patients, respectively. Multivariate logistic regression analysis revealed history of previous hospitalisation (Odds Ratio [Confidence Interval]) (2.49 [1.04–5.93]; $P = 0.04$), female gender (3.09 [1.45–6.56]; $P = 0.005$) and lymphocyte counts $>3210/\text{mm}^3$ (2.25 [1.06–4.75]; $P = 0.034$) were related with uncontrolled status in school-age children with asthma.

Conclusion: Awareness of risk factors related with uncontrolled asthma may alert physicians who deal with asthmatic school-age children and may help prompt and rational intervention in order to achieve proper asthma control.

586

Vitamin D levels in peripheral blood in patients with mild to moderate bronchial asthma

Torres, J^{1,2}; Martínez, M³; Chavez, E³; García, D⁴; Fabiano, F²; Hernández, J⁵

¹Pediatría, Hospital Vargas de Caracas, Caracas, Venezuela; ²Instituto Docente de Inmunodiagnóstico, Caracas, Venezuela; ³Inmunología, Hospital Militar Dr. Carlos Arvelo, Caracas, Venezuela; ⁴Inmunología, Hospital Militar Dr. Carlos Arvelo, Caracas, Venezuela; ⁵Centro Médico Fátima, Caracas, Venezuela

Background: Vitamin D plays a key role in the immune response generated by lymphocytes and antigen presenting cells, their role in regulating immune system cells has been recognised by the discovery of the vitamin D receptor (VDR) in almost all cells of the immune system, including T cells, B cells, neutrophils, macrophages and dendritic cells.

Method: For this we took 30 patients with mild to moderate asthma aged between 12 and 50 years of age, without severe associated pathologies and were asked measurement of serum IgE levels, vitamin D and spirometry were subsequently included in tables double-blind randomisation to receive vitamin D supplementation 1 g daily or placebo for 2 months. At the end they made new measurements of the above parameters were found in the group of

patients receiving medication and culminated the study (17 patients).

Results: There was a significant improvement in asthma symptoms according to forced expiratory volume in the 1st minute 58.7% of patients with the use of vitamin D supplementation with ap of 0.014 and increased peak flow ap of 0.047 which is statistically significant for these parameters, just as was evident in 76.4% of the same IgE levels decreased significantly after use of the drug with a statistical significance of $p = 0.017$. Similarly, patients in the control group who received placebo, also showed decreased serum IgE, but Tifenau index values (FEV1/FVC) significant differences in improvement with statistics $P = 0.028$ and 0.017 respectively. there was a significant improvement in asthma symptoms according to forced expiratory volume in the 1st minute 58.7% of patients with the use of vitamin D supplementation with ap of 0.014 and increased peak flow ap of 0.047 which is statistically significant for these parameters, just as was evident in 76.4% of the same IgE levels decreased significantly after use of the drug with a statistical significance of $p = 0.017$. Similarly, patients in the control group who received placebo, also showed decreased serum IgE, but Tifenau index values (FEV1/FVC) significant differences in improvement with statistics $P = 0.028$ and 0.017 respectively.

Conclusion: This opens a new field in the treatment of asthma patients because they can improve symptoms without the side effects of traditional therapy while suggesting new mechanisms in the pathophysiology of this disease as complex and common in our society.

590

Asthma control test: one size does not fit all

Caminati, M¹; Caimmi, C²; Schiappoli, M¹; Dama, A¹; Senna, G¹

¹Allergy Unit, Verona University Hospital, Verona, Italy; ²Rheumatology School of Specialization, University of Verona, Verona, Italy

Background: Pathophysiological variability of asthma (airflow limitation, airway inflammation and responsiveness) and the impact of comorbidities may account for the complexity of asthma monitoring and control achievement. A recent meta-analysis has confirmed Asthma Control Test (ACT) validity in assessing controlled and not well controlled asthma on the basis of Global Initiative for Asthma (GINA) definition of control, that does not include neither reversibility nor inflammation assessment, despite increasing evidence of their importance as hallmark of asthma,

partially unrelated to clinical outcomes. We aimed at exploring ACT validity according to a definition of control based on FEV1, reversibility test and airways inflammation.

Method: Control of asthma was assessed in 177 patients referring to our Unit. Control was defined as: FEV1 $\geq 80\%$ and negative reversibility test and FeNO < 50 ppb. Sensitivity, specificity, positive and negative predictive value of ACT in assessing asthma control on the basis of the definition above were examined. ACT performance in specific subgroups of patients was also evaluated by ROC curve analysis.

Results: ACT with cut-off score ≥ 20 shows good positive predictive value (83.5%) but low sensitivity (47.8%), specificity (66.7%), and negative predictive value (26.5%). Significant differences emerge when evaluating ACT performance in single subgroups of patients with different comorbidity and asthma severity. ROC curves analysis indicates that ACT in patients with mild intermittent rhinitis is more reliable (AUC: 0.714; $P < 0.05$) than in patients with nasal polyposis/chronic rhino-sinusitis (AUC: 0.176; $P > 0.05$). Considering asthma classification, the probability that ACT detects patients with uncontrolled asthma (true positive cases) is significantly higher in moderate persistent asthma subgroup than in mild persistent asthma one (OR 5.464; IC 95%: 2.5–11.9; $P < 0.05$).

Conclusion: According to a definition of asthma control based on functional parameters and airways inflammation assessment, ACT is reliable in detecting uncontrolled asthmatic patients but it does not show the same performance in identifying well controlled patients. According to ACT outcome, patients with mild persistent asthma seem to underestimate their pathology. Moreover, nasal comorbidity seems to significantly affect asthma perception and ACT performance. Our data suggest that different asthma phenotypes require different tools for an accurate follow-up.

592

Assessment of severity of asthma and impulse oscillometry

Cekerevac, I¹; Novkovic, G²; Novkovic, L¹; Lazic, Z¹; Petrovic, M¹; Mijailovic, Z³

¹Clinical Center Kragujevac, Medical Faculty Kragujevac, Clinical for Pulmonary Disease, Kragujevac, Serbia; ²RS Borromeo Mediglia Milano, Milano, Italy; ³Clinical Center Kragujevac, Medical Faculty Kragujevac, Clinical for Infective Disease, Kragujevac, Serbia

Background: The Impulse oscillometry (IOS) is non invasive test for measure mechanical impedance (respiratory resistance and reactance) of respiratory system

and does not require considerable patient cooperation compared to spirometric test. Aim of this study was to estimate clinical validity of IOS for the evaluation severity of asthma in adults patients.

Method: A group of 40 patients with asthma, which subdivided according to GINA into three groups was analyzed (Table 1). Asthma severity is classified on the basis of the intensity treatment required to achieve good asthma control.

Results:

Severity of asthma (n): Mild (14), Moderate (15), Severe (11)

Age: 38.4 ± 2.4 , 43.5 ± 3.6 , 48 ± 4.2

Male/female: 6/8, 7/8, 4/7

BMI: 25.7 ± 3.5 , 25.9 ± 3.8 , 28.3 ± 3.6

FEV₁:% 84.2 ± 16.3 , 69.2 ± 12.3 , 54.4 ± 15.2

R5%: 123.2 ± 4 , 179.1 ± 71 , 262.6 ± 86

R20%: 119.5 ± 32.7 , 148.5 ± 42.8 , 190 ± 54.4

Fres: 14.4 ± 5.6 , 23.2 ± 4.8 , 29.2 ± 5.5

The statistical significant highest values of FEV₁%, R5%, R20%, Fres were in the group patients with severe asthma. We found statistical significant negative correlation between FEV₁%, FVC% and R5%, R20%. Patients with moderate (R5% 179.1 ± 71.2 , R20%/148.5 ± 42.8) and severe asthma (R5%/262.6 ± 86.8 , R20%/190.0 ± 54.4) had dominant distal airway obstruction (resistance falls with increasing frequency).

Conclusion: Measurement of the following IOS parameters: R5%, R20%, Fres can be used for assessment of asthma severity, particularly in patients who cannot do correct spirometry due to uncooperation or severity of disease. Also, IOS can be used for estimation of small airway involvement in patients with asthma.

593

Clinical usefulness of impulse oscillometry (IOS) for the evaluation of stable asthma

Takahashi, K¹; Ito, T¹; Meguro, K¹; Yokota, M¹;

Kashiwakuma, D¹; Yoshida, S¹; Iwamoto, I¹

¹Asahi General Hospital, Department of Allergy and Clinical Immunology, Asahi, Japan

Background: IOS is a new noninvasive technique to assess lung function, but the interpretation of IOS data has not been well established. We thus determined the usefulness of IOS data for the evaluation of stable asthma.

Method: This is a single center retrospective evaluation of 258 stable asthma patients. We collected detailed patient characteristics, asthma control status, blood eosinophil counts, exhaled nitric oxide (FeNO) values, and lung function

values, and compared them with IOS data. Additionally, we performed spirometry and FeNO breath test (Sievers), followed by IOS (MasterScreen IOS, Carefusion Technologies) on the same day.

Results: (1) IOS values were strongly correlated with height, age, and lung function values of airflow obstruction. In particular, resonant frequency of reactance (Fres) was most strongly correlated with forced expiratory volume in one-second (FEV₁)/forced vital capacity (FVC) ratio (FEV₁/FVC), FEV₁/predictive FEV₁;%FEV₁, maximal flow rate at 50% of FVC (V50)/predictive V50;%V50 and maximal flow rate at 25% of FVC (V25)/predictive V25;%V25 ($P < 0.00001$, in all), and we obtained a regression line of FEV₁/FVC.

FEV₁/FVC (%) = $160.82 - 0.30\text{Age} - 0.38\text{Ht} + 0.083\text{Wt} - 0.44\text{Fres}$ ($R^2 = 0.476$)

(2) The duration of years before the start of inhaled corticosteroids and the disease duration were significantly correlated with the difference in respiratory resistance between 5 Hz and 20 Hz (R5-R20), impedance at 5 Hz (Z5), and resistance at 5 Hz (R5) (R5-R20: $P = 0.00011$, 0.00072 , Z5: $P = 0.0016$, 0.00090 , R5: $P = 0.0027$, 0.0018 , respectively).

(3) In assessment of the relationship between IOS data and treatment steps based on Japanese guideline (JGL2009), there was a significant difference in Fres between step 2 and 3 treatment groups ($P = 0.027$).

(4) Patients using bronchodilators showed significantly high Z5, R5, R20 and R5-R20 values ($P = 0.0047$, 0.0049 , 0.012 , and 0.034 , respectively).

Conclusion: It is suggested that in stable asthma, Fres is the best parameter of airflow obstruction, and R5, R5-R20, and Z5 that are more informative parameters with peripheral airway obstruction may reflect airway remodeling.

594

Experiences of living with asthma – a focus-group study with teenagers and parents to children with asthma

Jonsson, M¹; Egmar, A-C²; Kull, I³

¹Karolinska Institutet, Institute of Women and Childrens Health, Stockholm, Sweden; ²The Red Cross University College, Stockholm, Sweden; ³Department of Clinical Science and Education, Södersjukhuset, Stockholm, Sweden

Background: The goal for asthma treatment is that every individual, as far as possible, shall live without symptoms and exacerbations but many patients fail to achieve this goal. Moreover, patients and healthcare professionals sometimes have different perceptions of what is of importance to achieve well-being. These aspects need to be explored, from a patient per-

spective, to be able to develop good asthma care.

Aim: To describe teenagers with asthma and parents to young children with asthma experiences of living with asthma.

Method: Four focus groups interviews were performed, two in each group; parents to children 2–12 years with asthma, and teenagers with asthma. The data was tape-recorded and transcribed verbatim and analyzed qualitative according to systematic text condensation.

Results: The results were presented in three themes relevant to the participant's experiences of living with asthma; asthma management, limitations and needs. The experiences for teenagers and parents to children with asthma differed to some extent. The teenagers focus was mostly on how they managed the disease. They wanted to be like others and did not want to be seen differently because of the asthma. Teenagers with asthma often used different adaption strategies.

Parents to children with asthma emphasized on limitations and needs, expressed as feelings of frustration and anxiety, lack of knowledge and a need to meet professional competence both in health care and in school. Both teenager and parents expressed a need of support and understanding from healthcare professionals.

Conclusion: The experiences for teenagers and parents to children with asthma differed to some extent. Teenagers focus was on how to manage the disease and sometimes they endured the asthma symptoms in contrast to parents who more expressed limitation and needs.

Clinical implication: The health care professionals but also teachers in nursery school and school need an improved understanding about teenagers and parents experiences of how it is to live with an asthma disease in daily life. A more patient-centred education could be a way to gain this understanding.

596

Association between asthma and rhinitis prevalence, use of health resources, quality of life, and number of atopic sensitisations

Christoff, GC^{1,2}; Karova, EG³; Stoeva, IL⁴

¹Department of Health Economics, Medical University – Sofia, Faculty of Public Health, Sofia, Bulgaria; ²Allergy Outpatient Clinic, Tokuda Medical Centre, Sofia, Bulgaria; ³Faculty of Dental Medicine, Department of Conservative Dentistry, Medical University – Sofia, Sofia, Bulgaria; ⁴Faculty of Dental Medicine, Department of Allergology, Physiotherapy and Clinical Radiology, Medical University – Plovdiv, Plovdiv, Bulgaria

Background: Atopy, defined as at least one positive sensitisation to environmental

allergens is the main characteristic of allergic diseases. Its role as a risk factor for asthma, rhinitis and dermatitis is well known. On the other hand few data are available on the relation between atopy and social and economic determinants in people with asthma and allergic rhinitis. The objective of the current study is to assess association between asthma and rhinitis prevalence and atopic sensitisation together with the putative relation with changes in quality of life and use health resources.

Method: Patients, 225 men and women, aged 4–81 years consulting the outpatient clinic of a major private hospital in Sofia, because of a suspected IgE-mediated allergic disease were included. All participants completed a written questionnaire to define asthma and rhinitis prevalence, frequency of symptoms, quality of life and health costs. Patients underwent skin prick testing with 18 commercial allergen extracts using individual lancets (Alyostal Prick, 100 IR-IC/ml, Stallerpoint, Stallergenes, France; Cow milk prick 1000 BU/ml Bul Bio-NCIPD, Bulgaria). All test and reading procedures were in line with published practice guidelines.

Results: 72.7% of defined asthmatics are atopic. Non-asthmatics are with lower percentage of sensitisation – 56.7% ($P = 0.025$). Atopy is more frequent in patients with rhinitis than in these without – 70.4% vs 55% ($P = 0.018$). The most frequent sensitisation in asthmatics is that to four cereals – 35.7%, the least frequent – to *Cladosporium mix* – 1.8%. There is no difference between mean number of asthmatics sensitisations – 6.9 and non-asthmatics – 5.8 ($P = 0.095$) as well as in patients with and without rhinitis – 5.92 vs 5.88 respectively ($P = 0.067$). No correlation is found between quality of life indices in patients with asthma and rhinitis and number of sensitisations. The study does not show correlation between number of sensitisations and health resources use in asthmatics. Relative risk (RR) for asthma in sensitised people is 2.04 (1040–3990), $P = 0.038$, for rhinitis – 1.895 (1043–3445), $P = 0.036$. Age and sex are not asthma predictors. Rhinitis probability decreases with 60% after the age of 44 – RR 0.394 (0.160–0.968), $P = 0.042$.

Conclusions: The study demonstrates high rates of sensitisations to major inhalant and food allergen in asthmatics and patients with rhinitis. Sensitisation but not sex and age is a potent risk factor for asthma and rhinitis.

598

Predictor risk factors for recurrent wheezing in infants in a poor urban city in south Brazil

Pereira, MU¹; Ivancevich, JC²; Solé, D³; Mallol, J⁴; De-laBianca, AC⁵; Aranda, C³; Wandalsen, GF³; Rosário, N⁶; Chong, HJ⁶; Toledo, EC⁷; Oliveira, CM⁷; Moraes, LSL⁸; Takano, OA⁸
¹Catholic University of Rio Grande do Sul, Uruguaiiana, Brazil; ²Universidad del Salvador, Buenos Aires, Argentina; ³Federal University of São Paulo, São Paulo, Brazil; ⁴University of Santiago de Chile, Santiago, Chile; ⁵Federal University of Pernambuco, Recife, Brazil; ⁶Federal University of Paraná, Curitiba, Brazil; ⁷University of São José do Rio Preto, São José do Rio Preto, Brazil; ⁸Federal University of Mato Grosso, Cuiabá, Brazil

Objectives: To identify the predictor risk factors for recurrent wheezing in infants in the city of Uruguaiiana, RS, Brazil.

Methods: This is a cross-sectional study, part of the EISL (International Study of Wheezing in Infant). The parents or legal guardians of infants aged 12–15 months attending health centers for immunisation were interviewed and administered the EISL questionnaire (standardised and validated instrument) with questions on demographic characteristics, wheezing, respiratory infections and risk factors during the period between January 2008 and July 2010.

Results: Sampled infants ($n = 1.061$) had a mean age of 13.09 months and prevalence of wheezing during their first year of life of 28.6%. 10.4% had 3–6 episodes. They lived in a poor area of the city, with low maternal education level (60% had less than 8 years of formal education) with an income lower than US\$ 500 (99.8%). Significant differences were observed with respect to first cold younger than 3 months (OR: 3.2 95%CI: 2.16–4.73), eczema in infants (OR: 1.9 95%CI: 1.05–3.26), humidity in the home (OR: 1.9 95%CI: 1.27–2.98), pets before birth (OR: 1.5 95%CI: 1.03–2.27), and mother smoking (OR: 2.4 95%CI: 1.54–3.81).

Conclusions: The prevalence of wheezing among infants living in a poor area of Uruguaiiana is high. To identify the risk factors for recurrent wheezing in this low socio-economic population is mandatory for the establishment of preventive actions, early diagnosis of asthma and opportune treatment for this condition.

599

Assessment of compliance to treatment in asthmatic children

Can, C¹; Akkelle, E¹; Gökmez Özdemir, P¹; Yazıcıoğlu, M¹; Süt, N²
¹Department of Pediatric Allergy and Immunology, Trakya University Faculty of Medicine, Edirne, Turkey; ²Department of Biostatistics, Trakya University Faculty of Medicine, Edirne, Turkey

Background: Asthma is one of the most common chronic diseases in childhood, yet

compliance to treatment is reported to be 50% or less. The aim of our study was to evaluate the proper use of long-term asthma control and quick-relief medications, and to determine the compliance to treatment in asthmatic children.

Method: The study was conducted on 100 asthmatic children aged 6–18 years, monitored by the outpatient clinic of the Pediatric Allergy Department, Faculty of Medicine, Trakya University, Edirne. Patients with persistent asthma who had used their control medications at least for 30 days were included in the study. Long-term asthma control and quick-relief medications were prescribed, verbal and written information on the use of medications was given, and after one month patients and their families were asked to answer a questionnaire on drug use.

Results: The mean age of the patients (47% female) was 9.39 ± 2.76 years. All of the patients had persistent asthma. Seventy-three percent of the patients reported that they were using asthma controller medications regularly. The mean age of patients using preventive drugs regularly was 9.05 ± 2.5 years, with the mean age of patients using them irregularly 10.29 ± 3.26 years. This difference was statistically significant ($P = 0.047$). Regular use of preventive drugs did not differ between sexes. Seventy-seven percent of the patients with controlled asthma, 67.5% with partly controlled asthma, and 66.6% with uncontrolled asthma reported that they were using preventive drugs regularly. There was no difference in regular drug use between the groups regarding the level of asthma control. All of the patients who were recommended to use long-term asthma control medication identified their drugs correctly. Ninety-three percent of them described the dose accurately. There was no difference in compliance when a single drug or two drugs for prophylaxis were used. When they were asked about quick relief asthma medications, 62% of the patients reported salbutamol, 28% terbutaline, and 1% inhaled steroids as their quick-relief medication, while 9% of the patients did not know the name of their medication. Thirty-four percent of the cases correctly described the dose of their quick-relief medication.

Conclusion: The most important factor affecting regular use of preventive drugs in asthmatic children is age. This result suggests that children have more effective asthma treatment when it is under parental control.

Poster Session 12

The role of biomarkers in the management of asthma

600

Expression of ORMDL3 and CTLA4 genes in childhood asthma and their association with the antiasthmatic effect of inhaled corticosteroids

Berce, V¹; Kozmus, CEP²; Potočnik, U³

¹Department of Pediatrics, University Medical Center Maribor, Maribor, Slovenia; ²Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal; ³Center for Human Molecular Genetics and Pharmacogenomics, Faculty of Medicine, Maribor, Slovenia

Background: ORMDL3 and CTLA4 genes affect the lung immune responses, which are also the principal target of inhaled corticosteroids. Therefore we analyzed the interactions between ORMDL3 and CTLA4 genotype, gene expression and the effect of therapy with inhaled corticosteroids in childhood asthma.

Method: We analyzed a case-control cohort composed of 204 Slovenian children with mild to moderate persistent atopic asthma and 90 children with non-atopic asthma. Lung function was measured before and after 4–6 weeks of therapy with fluticasone dry powder. We genotyped functional polymorphisms CTLA4 CT60 and ORMDL3 rs2872507 and extended the study to analyze the effect of genotype and therapy on CTLA4 and ORMDL3 gene expression.

Results: Forced expiratory volume in 1 s (FEV₁) increased by 13.3% of predicted value after therapy in atopic subgroup of asthmatics with ORMDL3 rs2872507 AA genotype, compared to 4.9% increase in GG homozygotes ($P = 0.0176$). Median relative expression of ORMDL3 gene in asthmatics with AA, AG and GG genotype was 0.75, 1.05 and 1.21 respectively ($P < 0.0001$). Treatment with inhaled corticosteroids significantly increased median relative expression of ORMDL3 gene in atopic asthmatics from 0.88 to 1.21 ($P = 0.0032$). Regarding CTLA4 CT60 FEV₁ increased by 21.7% in atopic asthmatics with AA genotype compared to 5.8% increase in GG homozygotes ($P = 0.0067$). Median relative expression of full length CTLA4 isoform in asthmatics was 0.440, compared to 1.000 in healthy controls ($P < 0.0001$), and of soluble CTLA4 (sCTLA4) isoform in asthmatics was 0.580 compared to 1.040 in healthy controls ($P < 0.0001$). After treatment, the

sCTLA4 expression in asthmatics increased from 0.435 to 0.645 ($P = 0.049$).

Conclusion: CTLA4 and ORMDL3 gene expression is dysregulated in childhood asthma. Functional variants in ORMDL3 and CTLA4 genes are associated with the response to treatment with inhaled corticosteroids in children with atopic asthma, probably by their influence on gene expression. Our findings identify new potential biomarkers in asthma treatment and contribute to the understanding of the pharmacogenetics of corticosteroids in asthma.

602

Relationship of methacholine and mannitol responsiveness with serum levels of squamous cell carcinoma-related antigens in children with asthma

Baek, H-S¹; Lee, S-Y²; Lee, H-R²; Izuhara, K³

¹Department of Pediatrics, Hallym University College of Medicine, Seoul, Korea; ²Department of Pediatrics, Hallym University College of Medicine, Anyang, Korea; ³Saga Medical School, Saga, Japan

Background: TH₂ cytokines, which are known to be involved in the pathogenesis of allergic disorders, stimulate new synthesis of squamous cell carcinoma-related antigens (SCCA) in cultured human airway epithelial cells. Increased serum levels of SCCA have been observed in patients with allergic disorders such as atopic dermatitis and asthma. Effects of serum SCCA on bronchial hyperresponsiveness (BHR) have not yet been demonstrated in the human airway. The aim of this study was to address the relationship between serum SCCA levels and BHR in children with asthma.

Method: Seventy-nine children between the ages of 6 and 12 years were included and comprised the asthmatic group ($n = 54$) and the healthy control group ($n = 25$). We established new enzyme-linked immunosorbent assays (ELISAs) to specifically detect SCCA1 or SCCA2. Mannitol and methacholine provocation challenges were performed. The response to methacholine was expressed as a provocative concentration causing a 20% decrease in FEV₁ (PC₂₀). The response to mannitol was expressed as a provocative dose causing a 15% fall in FEV₁ (PD₁₅) and the response-dose ratio (RDR).

Results: The children with asthma had a significantly higher mean (\pm SD) level of SCCA1 than the controls (0.919 ± 0.599 vs 0.768 ± 0.261 ng/ml; $P = 0.039$). There were no significant differences in serum SCCA2 levels between the asthmatics and the controls (0.565 ± 0.254 vs 0.600 ± 0.168 ng/ml; $P = 0.265$). Serum SCCA1 levels were significantly correlated with mannitol PD₁₅ ($r = -0.507$, $P = 0.003$) and RDR to mannitol ($r = 0.332$, $P = 0.032$) but not with methacholine PC₂₀ ($r = -0.183$; $P = 0.272$). Serum SCCA2 levels were also significantly correlated with mannitol PD₁₅ ($r = -0.488$, $P = 0.005$) and RDR to mannitol ($r = 0.509$, $P = 0.001$) but not with methacholine PC₂₀ ($r = -0.227$; $P = 0.170$).

Conclusion: Serum SCCA1 levels increased in asthmatic children. Both SCCA1 and SCCA2 levels were related to BHR to mannitol but not to methacholine in asthmatic children.

603

Detection of IgE and IgG anticitrullinated protein antibodies in asthmatic patients

Garriga Baraut, T¹; Labrador-Horrillo, M¹; Luengo, O¹; Guilarte, M¹; Sala-Cunill, A¹; Cardona, V¹

¹Hospital de la Vall d'Hebron, Allergy, Barcelona, Spain

Background: Persistent inflammation of the airway is considered the main feature of asthma. Nitric oxide (NO) is an inflammatory mediator which is an easy, repeatable and non-invasive marker of eosinophilic bronchial inflammation formed in biological systems from L-arginine and oxygen by the nitric oxide synthase (NOS). NOS converts L-arginine into NO and L-citrulline. Citrulline can appear in peptides against which anticitrullinated protein antibodies (ACPAs) have been described. Allergic asthma is associated with increased exhaled nitric oxide (FeNO) measurements. Therefore, asthmatic patients with increased FeNO measurements may also develop IgG and IgE ACPAs. Hence, the aim of this study was to determine if IgG and IgE ACPAs are present in sera of atopic asthmatic patients.

Method: Prospective cohort study. A blood sample was obtained from atopic asthmatic patients whose levels of FeNO

were ≥ 50 parts per billion (ppb) ($n = 37$) and from atopic non asthmatic patients with levels of FeNO ≤ 50 ppb (control; $n = 6$). All patients were tested for IgE and IgG ACPAs using an ELISA technic (Phadia, Germany) on the ImmunoCAP 100 instrument according to the manufacturer's instructions.

Results: Forty-three patients (25 female and 18 male) with a mean age of 32 years were included. IgG-ACPs were detected in only one asthmatic patient and in none of the atopic controls. On the other hand, IgE-ACPs were detected in between 43.2% ($n = 16$) and 75% ($n = 27$) of the asthmatic patients depending on the cut-off point used and in one atopic non-asthmatic control. Regarding relationship between IgE-ACPs and FeNO measurements, patients with asthma with FeNO levels between 100 and 150 ppb had increased IgE-ACPs values ($P < 0.05$).

Conclusion: This is first study in which the presence of IgE-ACPs has been described in patients with extrinsic asthma and increased FeNO measurements. However, in humans, the potential role of ACPs in asthmatic patients has not yet been elucidated.

607

Identification of serum biomarkers in patients with asthma and other allergic diseases in Venezuela

De Sanctis, JB¹; Larocca, NE²; Moreno, D²; Garmendia, J¹; Martín-Rojo, J¹; Tálamo, C³

¹Instituto de Inmunología, Universidad Central de Venezuela, Caracas, Venezuela; ²Cátedra de Patología General y Fisiopatología, Universidad Central de Venezuela, Caracas, Venezuela; ³Servicio de Neumonología, Universidad Central de Venezuela, Caracas, Venezuela

Background: Asthma is a chronic inflammatory respiratory disease with a high prevalence around the world. Several biomarkers (particular induced sputum analysis, pulmonary function tests as spirometry and the fraction of nitric oxide in exhaled air) have been tests to assess disease activity and predict potential response to therapy. Currently, the value of serum biomarkers remains controversial. Few studies have evaluated serum biomarkers in association with asthma and other allergic diseases in our mixed-race population. The aim of this study was explore the association between serum concentrations of several cytokines and other soluble mediators in subjects with asthma and other allergic diseases in Venezuelan population.

Method: We measured 20 serum biomarkers in 165 subjects with asthma, 65 allergic subjects without asthma and 140 healthy controls, using serum samples and

different commercial kits based in enzyme immunoassay for detect follows molecules: IgG and IgE antibodies for *Toxocara canis* and *Strongyloides stercoralis*; C-Reactive Protein (CRP); sCD54, sCD62L, sCD154; nitric oxide (NO) oxidation products: nitrites, nitrates and nitrotyrosine; proinflammatory cytokines: Tumor Necrosis Factor (TNF), IL-6 and IL-8; metalloproteinases: MMP-2, MMP-3 and MMP-9; metalloproteinases inhibitors as tissue inhibitor of metalloproteinase-1 (TIMP-1); alpha-1 antitrypsin; malondialdehyde, glutathione and antioxidantized LDL antibody (anti-oxLDL).

Results: We observed significant differences between asthmatic patients and atopic and controls subjects; asthmatic patients had higher levels of inflammatory proteins (CRP, TNF), activation soluble markers (sCD54, sCD62L and sCD154), stress oxidative molecules (nitrotyrosine, malondialdehyde and anti-oxLDL) and metalloproteinases (MMP-3 and MMP-9) ($P < 0.01$). In the group with asthma, we also observed glutathione, nitrites and nitrates levels lower than controls ($P < 0.01$).

Conclusion: This study supports the hypothesis that an imbalance between oxidant-antioxidant and proteases-antiproteases are associated to the oxidative stress which plays a significant role in progression and severity of asthma. In the future similar studies could evaluate the utility of these biomarkers in clinical practice for to make more accurate diagnoses, identify individual's disease phenotype and create personalized therapies.

609

Correlation between asthma and plasma omega fatty acids

Yoon, HR¹; Ahn, YU¹; Yeo, SH²; Kwon, SH³; Lee, SH³; Lee, KR³; Kim, SH⁴; Chang, SI⁵; Yoon, HJ⁴

¹College of Pharmacy, Duksung Women's University, Seoul, Korea; ²AB Applied Biosystems, Seoul, Korea; ³Seoul Medical Science Institute, Seoul, Korea; ⁴Internal Medicine, Hanyang University College of Medicine, Seoul, Korea; ⁵Sungae General Hospital, Seoul, Korea

Background: The purpose of study was to conduct possible value of omega-3 fatty acid supplementation in asthma was sparked by Horrobin's hypothesis that the low incidence of asthma in Eskimos stems from their consumption of large quantities of oily fish, rich in omega-3 fatty acids. As a first preliminary research, this study conducted correlation between asthma and plasma omega fatty acids in asthma patients for the evidence for the preventive, health effects of omega-3 fatty acids.

Method: A sensitive, de novo analytical method was developed for the direct deter-

mination of omega fatty acids in plasma without complicate preparation by MS/MS. Five microlitre of α -linolenic acid (ALA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), arachidonic acid (AA) and eicosapentaenoic acid (EPA) (50 ng/ml each) of standard solutions were injected through guard column into the source of negative ion electrospray MS/MS was set with specific ions (ALA; m/z 277.2 \rightarrow 233.1, DHA; m/z 327.2 \rightarrow 283.3, DPA; m/z 329.1 \rightarrow 285.3, AA; m/z 303.3 \rightarrow 259.2, EPA; m/z 327.2 \rightarrow 283.3) using multiple reaction monitoring mode. Calibration curve against 5 omega fatty acids studied showed correlation coefficient of more than 0.98 in the range of 5–1000 ng/ml ($n = 3$) on plasma matrix. Plasma concentration of omega fatty acids was quantified using the new method.

Results: Plasma concentration of 5 omega fatty acids for asthma group ($n = 20$) were approximately less than 20% to the healthy control group ($n = 100$). DHA; less than 20% of normal, EPA and AA; less than 16.6% of normal, ALA; less than 30% of normal, DPA; less than 18.8% of normal.

Conclusion: This result speculates that deficient concentration of plasma omega fatty acids in asthma patients might one of the causes of exacerbation of asthma.

610

Atopy, exhaled nitric oxide and body mass index in patients with asthma

Sergeeva, G¹; Leshenkova, E¹; Kozireva, L¹; Rebrova, S¹; Emelyanov, A¹

¹North-Western Medical University, Saint-Petersburg, Russia

Background: We examined the relationship between body mass index (BMI) and exhaled NO (FeNO) as a marker of airway inflammation in patients with atopic vs non-atopic asthma.

Method: In 116 out-patients (aged 18–81 years, mean age 52 years, 28% males) with asthma spirometry and body mass index (BMI) were measured. Skin prick tests or serum specific IgE to common inhalant allergens were used to assess atopic status. FeNo was measured by Logan 4100 analyzer. Quality of life and asthma control were assessed by using Russian version of St. George's Respiratory Questionnaire (SGRQ) and asthma control test (ACT-test).

Results: Sixty-seven percent were atopic. Only 4% of patients with asthma were underweight, 30% had normal BMI, 37% and 29% were overweight and obese, correspondingly. BMI, severity of asthma, asthma control and quality of life did not

differ in atopic and nonatopic asthmatics. In atopic steroid-naïve asthmatics FeNO was higher than in nonatopic (50.4 vs 14.9 ppb, $P < 0.01$) and in atopic subjects treated with ICS (50.4 vs 30.6 ppb, $P < 0.05$). We found an inverse relationship between BMI and FeNO ($r = -0.36$, $P < 0.05$) in patients with atopic asthma. There was a significant correlation between BMI and FeNO ($r = 0.51$, $P < 0.05$) in non-atopic asthmatics.

Conclusion: Atopy, overweight and obesity are common in asthmatic patients. There was a significant correlation between BMI and FeNO according to atopic status that may reflect different types of airway inflammation in asthmatic patients.

611

E-cadherin as an epithelial barrier protein: relation to atopy in children with asthma

Turkeli, A¹; Yilmaz, O¹; Taneli, F²; Dinc Horasan, G³; Toprak Kanik, E⁴; Kizilkaya, MH⁴; Gozukara, C²; Yuksel, H¹

¹Celal Bayar University, Pediatric Allergy and Pulmonology, Manisa, Turkey; ²Biochemistry, Celal Bayar University, Manisa, Turkey; ³Biostatistics, Celal Bayar University, Manisa, Turkey; ⁴Department of Pediatrics, Celal Bayar University, Manisa, Turkey

Background: Epithelium forms the most important physical barrier of airway. It is not permeable due to the tight junctions (TJ) between cells. E-cadherin plays an important role in development and maintenance of epithelial cell-cell interaction in the airway. Tight junction proteins ZO-1, occludin and claudin delocalise and TJs are distorted if E-cadherin is not adequately expressed. Epithelial permeability is significantly increased in asthma and allergens and irritants reach basal layer more easily. Exhaled breath condensate (EBC) has been shown to be a reliable noninvasive method to show biomarkers specific to the airway in children with asthma in various studies.

We measured soluble E-cadherin in EBC in children with asthma to demonstrate epithelial damage.

Methods: We enrolled 39 atopic and 39 nonatopic children with asthma in this study. Atopic group consisted of children who had skin prick test (SPT) positivity to at least one of the aeroallergen extracts with protease activity (mite, mold, olea) Nonatopic group consisted of children who had negative SPT. Exhaled breath condensate was obtained for measurement of soluble E-cadherin from all children enrolled. Soluble E-cadherin levels were measured with the ELISA method.

Results: Age, gender and asthma severity was not significantly different among the

two groups. Mean levels of soluble E-cadherin in EBC samples from children with atopic and nonatopic asthma were 0.25 (0.29) vs 0.11 (0.09) ng/ml respectively ($P = 0.08$).

Conclusion: Soluble E-cadherin levels were higher in atopic children with asthma compared to the nonatopic ones though the difference was statistically insignificant. This may indicate that E-cadherin slough off from damaged epithelium is higher in atopic children with asthma compared to nonatopic children with asthma due to allergen exposure.

612

Overview on plasma cortisol levels in asthma and allergy diseases at Manado – Indonesia

Surachmanto, EE^{1,2}

¹Internal Medicine, Sam Ratulangi University, Manado, Indonesia; ²Internal Medicine, RSUP Prof. R.D. Kandou Hospital, Manado, Indonesia

Background: Cortisol is a hormone that plays a role in regulating the immune system. Primarily produced in the adrenal cortex, cortisol secretion is regulated by adrenocorticotrophic hormone (ACTH) and shows profound circadian rhythmicity and responsiveness to physical and psychological stressors. Recent studies have found lower levels or reduced responsiveness of endogenous cortisol to stress in asthma and allergy. Activation of the hypothalamic-pituitary-adrenal (HPA) axis with glucocorticoid release is one characteristic feature of the acute response to stress. However, the association of endogenous cortisol levels or responses with airway inflammatory activity in stress remains unexplored. The aim of this preliminary study was to see the level of morning plasma cortisol in patients with allergic diseases.

Method: Seventy – four chronic allergic patients (atopic dermatitis, atopic asthma, rhinitis allergic, chronic atopic urticaria, etc.), ranging 1–78 years old. All patients that entered the study were never used or had discontinued oral steroid at least one month. Blood sample is collected at 8 am and serum cortisol is examined by electrochemiluminescence immunoassay (ECLIA) method.

Results: There were 74 chronic allergic patients consisting of 40 women (54%), 34 men (46%). The allergy diseases were dermatitis (35 cases), asthma (22 cases), rhinitis (7 cases), chronic urticaria (5 cases), drug allergy (2 cases), food allergy (1 case) and angioedema (2 cases). Data of the cortisol was performed in the percentage of the value from the upper limit of normal

cortisol level. Only 8 patients (10.8%) have normal cortisol level, 66 patient (89.2%) were below 50% of upper cortisol level, 17 (23%) patients were below normal range of cortisol level.

Conclusion:

- 89.2% of chronic allergic patients have cortisol values lower than 50% from normal upper limit value.
- Blood sample should be taken earlier at 6.00 am on awakening to have a more appropriate values
- More sample is needed

613

Clinical characteristics and treatment outcome related with sputum eosinophilia in Korean asthmatic patients

Choi, BW¹; Yoo, J-H²; Jung, J-W¹; Choi, J-C³; Shin, J-W³; Kim, J-Y³; Park, I-W³; COREA (Cohort for Reality, Evolution of adult Asthma) Research Group

¹Department of Internal Medicine, Division of Respiriology and Allergy, Chung-Ang University Hospital, Seoul, Korea; ²Department of Internal Medicine, Division of Respiriology, KyungHee University Hospital at Gangdong, Seoul, Korea; ³Department of Internal Medicine, Chung-Ang University Hospital, Seoul, Korea

Background: Allergic diseases including bronchial asthma can develop in individuals who are sensitised to indoor or outdoor allergens, and subsequently induce immunologic hypersensitivity reactions. Sensitivity to fungal allergens has been recognised as a risk factor for the development of asthma and may be related to persistent asthma, severe asthma, and potentially fatal asthma exacerbations.

We conducted this study to evaluate baseline clinical characteristics and treatment responses of asthmatics with and without atopy and atopy to fungus in Korean asthma patients.

Method: The patients with bronchial asthma who underwent skin prick test at baseline were selected from COREA cohort study population. Skin prick tests were performed using 11 inhalant allergens including Dermatophagoides pteronyssinus, D. farinae, tree pollen mixture, grass pollen mixture, ragweed, mugwort, cockroach, Alternaria, Aspergillus, cat and dog. Lung function was evaluated every 12 months during 36 months follow-up period. The subjects received the treatment guided by symptoms and spirometry according to Global Initiative for Asthma recommendations.

Results: The mean age in atopic asthma ($n = 728$) and non atopic asthma ($n = 1027$) was 40.3 and 54.8 ($P < .05$). FEV1 in non atopic asthma was significantly lower than in atopic asthma (79.2% vs 83.5%, $P < .05$). Paired T test of FEV1% between baseline and 12, 24, and

36 months of atopic asthma and non atopic asthma presented significant improvement after treatment (83.5%, 88.4%, 88.5%, 88.2% and 79.2%, 83.3%, 81.6%, 83.8%, respectively $P < .0001$). Among patient with atopy, chronologic changes of FEV1% compared with baseline FEV1% in fungal sensitisation group were not significant in 12, 24 and 36 months (81.4%, 85.5%, 83.1%, 77.4%, $P > .05$) unlike atopy to other than fungus group (83.6%, 88.5%, 88.7%, 88.8%, $P < .001$).

Conclusion: Asthma with atopy to fungus showed poor treatment outcome compared with asthma without fungal allergy in 3 years follow-up of Korean asthma cohort population.

615

Nasal challenge with 50 mgs of L-aspirin for diagnosis of ASA exacerbated respiratory disease

Reyna, JM¹

¹Allergy and Clinical Immunology, Zambrano Hellion Medical Center, Monterrey, Mexico

Background: The ASA exacerbated respiratory disease is an aggressive and continuous inflammatory disease of the airways, combined with exacerbation of asthma and rhinitis attacks, after ingestion of ASA and most of the NSAIDs. The diagnosis can be established with provocation test using increasing doses of ASA. Oral challenge is the most commonly performed, and has the higher sensitivity. (1)

Method: We assigned three groups of 20 patients. Group 1 were patients with AERD, group 2 were patients with asthma and nasal polyps, and group 3 were healthy volunteers. All the patients suspended the asthma medication before the nasal challenge and performed an initial spirometry and rhinomanometry, with at least an FVC in 80% and a 200 ml of nasal flow.

We administrated 12.5 mgs of L-aspirin in each middle tourbinate and 30 min after we performed another spirometry and rhinomanometry, the nasal challenge was considered positive if the FEV1 felt down 20% or more or the nasal flow decreased 40% or more, if not, the patients received another 25 mgs of L-aspirin, and 30 min later the patients performed another spirometry and rhinomanometry. The nasal challenge length was 4 h, and none of the patients had a severe reaction.

At the same time we recorded the clinical changes in the patients: nasal blockade, change in the color of the turbinates, sneeze, nasal pruritus, and rhinorrhea. We gave 1–3 points depending on the severity

of the change, and considered it positive if it scored 10 or more points.

Results: With the clinical changes we had a sensibility of 38.2% and a specificity of 96.2% with a positive predictive value of 0.93 and a negative predictive value of 0.54 and 95 interval of confidence. If the FEV1 felt down at least 20% or more, it had a sensibility of 50% and a specificity of 96%, a PPV of 0.94 and NPV of 0.59, and if the nasal flow felt down at least 40%, it had a sensitivity of 73.5% and a specificity of 96%, a PPV of 0.96 and NPV of 0.73, and if we had at least 1 positive result of the three parameters we had a sensitivity of 85% and a specificity of 96%, with a PPV of 96.7 and a NPV of 0.83.

Conclusion: The intranasal challenge is a safe and practical study for the diagnosis of AERD, and has a sensitivity and specificity similar to the oral challenge without the hospitalisations and the risk of severe reactions.

616

Urinary leukotriene E4 levels in atopic and non-atopic pre-school children with virus induced asthma: a potential marker of atopic, virus induced asthma phenotype?

Marmarinos, A¹; Saxon-Papageorgiou, P²; Manousakis, E²; Tsentidis, C¹; Cassimos, D³; Doxara, A⁴; Paraskakis, I⁴; Gourgiotis, D¹

¹Laboratory of Clinical Biochemistry – Molecular Diagnostics, 2nd Pediatric Clinic, Athens University Medical School, Athens, Greece; ²Department of Allergy, 2nd Pediatric Clinic, Athens University Medical School, Athens, Greece; ³Pediatric Department, General University Hospital of Alexandroupoli, Alexandroupolis, Greece; ⁴Department of Clinical Microbiology, 'P & A Kyriakou' Children's Hospital, Athens, Greece

Aim: To determine the levels of urinary leukotriene E4 (U-LTE4) in atopic and non-atopic pre-school children with virus induced asthma, in an effort to assess the relationship of urinary LTE4 to particular asthma phenotype in this age.

Method: Levels of urinary leukotriene E4 were measured by means of Enzyme Immunoassay (EIA) and the results were expressed in pg/mg of secreted Creatinine in 96 pre-school children (mean 3 years) with virus induced asthma, 52 atopic and 44 non-atopic, during exacerbation and in remission. Atopy was determined by specific serum IgE measurement and skin prick test to a panel of common allergens. Urinary LTE4 was also measured in 36 age-matched, non-asthmatic, non-atopic control children. The results were analyzed using the non-parametric Mann-Whitney U test.

Results: During exacerbation, U-LTE4 was significantly higher in all children, atopic and non-atopic, with virus-induced

asthma in comparison to A. Remission: 642.20 ± 268 vs 399.45 ± 204 , P value < 0.001 and B. Controls: 642.20 ± 268 vs 271.39 ± 83 P value < 0.001 . Atopic patients demonstrated significantly higher levels of U-LTE4 in relation to non atopic, both during exacerbation (872.13 ± 246 vs 613.15 ± 150 P value = 0.0013) and in the remission phase (507.59 ± 182 vs 283.59 ± 160 P value < 0.001). During remission, a highly significant difference of U-LTE4 was found to persist when controls were compared to atopic patients: 271.39 ± 83 vs 507.59 ± 182 P value = 0.002 but not when compared to non atopic ones: 271.39 ± 83 vs 283.59 ± 160 P value = 0.432.

Conclusion: Urinary leukotriene E4 (U-LTE4) is a strong indicator of virus induced asthma exacerbation in pre-school children, more so in atopics. Increased basal levels of urinary LTE4 occur only in atopics. This suggests a potential role of urinary LTE4 as a marker of atopic, virus induced asthma in pre-school children.

617

Effect of aspirin desensitisation on cytokine release from T-lymphocytes and plasma lipoxin levels

Aksu, K¹; Kurt, E²; Alataş, Ö³; Gülbaş, Z⁴

¹Department of Chest Diseases, Division of Allergy and Clinical Immunology, Ankara Numune Training and Research Hospital, Ankara, Turkey; ²Department of Chest Diseases, Division of Allergy and Clinical Immunology, Eskişehir Osmangazi University, Eskişehir, Turkey; ³Department of Biochemistry, Eskişehir Osmangazi University, Eskişehir, Turkey; ⁴Department of Haematology, Eskişehir Osmangazi University, Eskişehir, Turkey

Background: This study aims to evaluate the release of cytokines from CD4⁺ and CD8⁺ T-lymphocytes and plasma lipoxin levels in aspirin-exacerbated respiratory disease (AERD) and to observe the effect of aspirin desensitisation on these cytokine and lipoxin levels.

Method: Intracellular IL-4, 5, 10 and IFN- γ release from CD4⁺ and CD8⁺ T-lymphocytes and plasma lipoxin-A4, 15-epi-lipoxin-A4 were studied in 23 patients with AERD and 17 patients with aspirin tolerant asthma (ATA) who had been diagnosed and followed-up in allergy clinic between 2009 and 2012 and 16 aspirin tolerant healthy controls. For clinical evaluation of the patients spirometry, skin prick test, asthma control test, nasal symptom scores and smell scores were also assessed. Fourteen eligible AERD patients underwent aspirin desensitisation treatment and in this group intracellular cytokine release from T-lymphocytes and plasma lipoxin levels together with clinical assessment

were reevaluated after aspirin desensitisation followed by one-month daily aspirin treatment.

Results: Among the intracellular cytokines released from CD4⁺ and CD8⁺ T-lymphocytes CD4⁺IL-10 levels were significantly higher in AERD patients compared to healthy controls and CD4⁺IFN- γ levels were significantly higher in AERD patients and ATA's compared to healthy controls. Plasma lipoxin-A4 and 15-epi-lipoxin-A4

levels were similar among the three study groups. In AERD patients undergone aspirin desensitisation followed by 1-month daily aspirin treatment the clinical parameters including asthma control test, nasal symptom scores and smell scores improved and CD4⁺IFN- γ levels decreased significantly. No significant change in lipoxin levels was recorded. CD4⁺IFN- γ and CD4⁺IL-10 levels in AERD patients after

1-month daily aspirin treatment were similar with the levels in healthy controls.

Conclusion: This study confirms that aspirin desensitisation treatment is an effective therapeutic option in patients with AERD and suggests that IFN- γ and IL-10 release from CD4⁺ T-lymphocytes might be related to pathogenesis of AERD and mechanism of action of aspirin desensitisation treatment.

Innovative developments in hereditary angioedema

618

The first case diagnosed as both type III hereditary angioedema and familial Mediterranean fever

Buyukozturk, S¹; Gelincik, A¹; Demirturk, M¹; Unal, D¹; Colakoglu, B¹; Uyguner, Z²; Karaman, V²

¹Department of Internal Medicine, Division of Allergy, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey; ²Department of Medical Genetics, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey

Background: Hereditary Angioedema (HAE) and Familial Mediterranean Fever (FMF) are inherited diseases which have not been reported together before. We present here the clinical and molecular findings of a female patient with the concurrent occurrence of Type III HAE and FMF.

Case report: A 23 year-old patient experiencing angioedema abdominal pain and arthralgia attacks without fever for 5 years was referred. Although serum inflammation markers were in normal range in attack-free periods, they couldn't be analyzed during attacks. Her attacks occurred monthly in association with menstrual periods. Her mother and sister had similar but milder symptoms. C1 INH level was 21 (N: 21–39 mg/dl), C4:8.21 (10–40 mg/dl), C1 INH function: 59 (N:60–130%). Colchicine 1.5 mg per day was initiated empirically. At the end of 1 month attacks of abdominal pain, arthralgias were lessen, although angioedema continued. Therefore danazole 200 mg/day was initiated. Two months later, at her next visit, all of her symptoms were ceased while she was on danazole and colchicum dispert daily. Sequencing of exon 8 on chromosome 11 didn't reveal any mutations which had been described in Type I and Type II hereditary angioedema. Sequencing of F12 revealed homozygous c.-4T>C in the 5' UTR region of the gene. Targeted mutation analysis of the patient by sequencing the selected exons (2, 3, 5, 10) of marenstrin-encoding fever gene (MEFV gene) (NM_000243.2; NP_000234.1) which was shown to be related with FMF, revealed homozygous alteration of c.605G>A in exon 2 and heterozygous alteration of c.2230G>T in exon 10.

Conclusion: Association of homozygous c.-4T>C in the 5'UTR region of the F12 gene and variation in factor XII level in plasma

was reported in the literature. It is already revealed that gain of function mutations in F12 gene causes HAE III by increasing factor XII level in plasma. This genetic alteration, familial nature of the symptoms and treatment response to danazole suggest that our patient has a Type III HAE. To our knowledge this patient is the first case having both HAE and FMF. Since little is known for the genetic analyses of Type III HAE, this patient may possibly represent more than a coincidence.

619

Type I and II hereditary angioedema: clinical characteristics and treatment response with human C1-inhibitor (hC1-INH) in a French cohort

Bouillet, L^{1,2}; Boccon-Gibod, I^{1,2}; Launay, D^{2,3}; Laurent, J^{2,4}; Floccard, B^{4,5}; Martin, L^{2,6}; Kanny, G^{2,7}; Allaert, FA⁸; Catovic, H⁹; Fain, O^{2,10}; Gompel, A^{2,11}; Pagnier, A^{2,12}

¹Internal Medicine Department, Grenoble University Hospital, Grenoble, France; ²National Reference Centre for Angioedema, Grenoble, France; ³Internal Medicine Department, Lille 2 University, Lille, France; ⁴Hôpital Pompidou and Institut Pasteur, Paris, France; ⁵Intensive Care Unit, Edouard Herriot University Hospital, Lyon, France; ⁶Dermatology Department, Angers University Hospital, Angers, France; ⁷Allergology Unit, Nancy University Hospital, Nancy, France; ⁸DIM, CHRU Dijon, Dijon, France; ⁹CSL Behring, Paris, France; ¹⁰Internal Medicine Department, Jean Verdier University Hospital, AP-HP, Bondy, France; ¹¹Gynecology Department, Cochin University Hospital, AP-HP, Paris, France; ¹²Grenoble University Hospital, pediatrics, Grenoble, France

Background: Type I and II hereditary angioedema (HAE) are characterised by a reduced production or functional deficiency of C1-Inhibitor (C1-INH), respectively. Here we report clinical data and treatment response with hC1-INH in a French population of patients with HAE-I/II.

Method: We conducted an analysis of patients with HAE-I/II included in the National Angioedema Reference network from 2007 to December 2012. Data were obtained from the recently established CohortBeRinertAngioedème (COBRA) registry study, which aims to collect information on all C1-INH-treated patients with HAE throughout France. The analysis included retrospective data extracted from patients' medical records and prospective data directly recorded in the elec-

tronic registry. Data on treatment response were only available for documented attacks after COBRA enrollment.

Results: Eighty-nine patients (women: 67.4%) with HAE-I/II treated with human C1-INH for an average of 4.3 ± 4.9 years were included. At inclusion in the COBRA registry, mean age was 35.5 ± 18.2 years old. The first HAE attack occurred at 13.0 ± 11.3 years, and HAE diagnosis was made at 18.2 ± 14.1 years. Mean age at first hC1-INH treatment was 31.4 ± 17.4 years. Fifty-eight percent of patients received tranexamic acid and 44% danazol. During the last year before inclusion in the COBRA registry, patients had a mean 5.5 ± 6.3 attacks. Among these attacks, 1.0 ± 2.8 were treated with human C1-INH while 19% of patients received human C1-INH for prophylaxis. The registry allowed to describe 179 attacks, 164 were retrospective and 11 were prospective (4 missing data). Localisation was abdominal in 40.2%, facial in 34.9%, laryngeal in 32.5% and peripheral in 18.9% of patients. They were moderate (43.5%) or severe (54.0%) with associated symptoms (23.5%), essentially nausea/vomiting. A trigger event was described in 24.0% of patients, involving stress or trauma. Treatment required 1000 IU in 72.2% of cases and 1500 IU in 15.2%. After hC1-INH infusion, HAE symptoms began to improve within 1 h in 42.5% of patients and disappeared within 24 h in 56.9% of patients. Twenty-eight patients (89.3% older than 18) were prophylactically treated (once in 64.3% and twice in 28.6%), mainly before surgery (39.6%) or dental work (27.9%). Prevention was successful in 97.1% of cases.

Conclusion: The COBRA registry provides the opportunity to describe HAE-I/II patients systematically treated with human C1-INH and monitor its efficacy, either in attack treatment or prevention.

620

Oestrogen-independent type III hereditary angioedema in a 10-year-old boy

Bergmann, MM¹; Caubet, J-C¹; Eigenmann, PA¹
¹Department of Pediatrics, University Hospital of Geneva, Allergy Unit, Geneva, Switzerland

Background: Hereditary angioedema (HAE) is associated with an either quantitative (Type I) or qualitative (type II) deficiency of the protein C1 esterase inhibitor (C1-INH). Recently HAE type III with normal C1-INH activity, occurring in woman and influenced by oestrogens has been described. HAE represent a rare but challenging differential diagnosis of acute angioedema, particularly in children. Its presentation can be similar to other more frequent causes of angioedema due to histaminic release.

Results: We report of a 10-year-old boy who presented with subacute edema of the eyelids, the right hand, the left foot and the penis as well as dysphagia and epigastric pain. He also presented itchy urticarial lesions on both hands and feet for about 10 days. No triggering drug, food or infection could be identified. Six years prior to this episode, he experienced a similar attack of angioedema while taking ibuprofen for a viral upper airway infection. Family history revealed maternal episodes of angioedema in childhood. The sister of the mother had also described similar symptoms. No other family members were affected. Physical examination was remarkable for pale edema of the penis, the right hand and left foot, the uvula and the anterior peri-tonsillar pillars. Symptoms rapidly disappeared after injection of icatibant with complete regression after about 3–5 h. Laboratory findings during acute episode showed normal serum tryptase, fraction 3 and 4 of the complement levels, CH50 and C1-INH function. Exploration of the bradykinin metabolism showed an increased spontaneous activity of kinin-producing kininogenases. None of the two known mutations (Thr328Lys/Arg) of factor XII (FXII) gene associated with angioedema type III were present.

Conclusion: A diagnosis of hereditary angioedema type III was suspected in presence of a normal initial work-up including C1-INH activity and C4 level. The pathogenesis is thought to be mainly due to a gain of function of the kinin-producing kininogenases. Only a few pediatric cases of type III HAE have been published so far, all females. This is the first pediatric male case of type III HAE published, showing that angioedema with normal C1-INH might also occur in pediatric population, independent on gender and hormonal influence.

621

Nanofiltrated C1-esterase-inhibitor in the prophylactic treatment of hereditary angioedema

Strassen, U¹; Bas, M¹; Hajdu, Z¹; Buchberger, M¹
¹Klinikum rechts der Isar der Technischen Universität München, Hals-Nasen-Ohrenklinik und Poliklinik, Munich, Germany

Background: Hereditary angioedema is a rare genetic disease induced by a deficiency of the C1 esterase inhibitor. It is associated with increased bradykinin levels leading to raised vascular permeability. Consequently patients develop recurrent edema of (sub-) cutaneous and mucosal structures. Current therapy consists of substitution of C1-esterase-inhibitor concentrate or B2 bradykinin receptor antagonists. Prophylactic therapy is recommended in severe cases.

Method: On demand therapy with Berinert P 1500 units and/or Firazyr 30 mg was compared with prophylactic therapy in three patients. Prophylactic therapy consisted of a dose of 1000 units Cinryze twice weekly. Cinryze was self-administered by patients intravenously. Patient diaries and patient files were used to compare symptom control, quality of life, adverse events and administered dosage per month.

Results: We could show that prophylactic therapy with Cinryze led to significant improvement in symptom control and quality of life. Drug dosage could be reduced in both cases. Self-administration was rated well by both patients. Except for a local rash after injection of Firazyr no adverse events were reported for either of the drugs.

Conclusion: Long term prophylactic therapy with Cinryze for severe forms of hereditary angioedema proofed to be safe, cost-efficient and effective.

622

Hereditary angioedema patient experience with short term prophylaxis: responses to an on-line questionnaire survey

Bernstein, JA¹; Singh, U¹; Wilmont, J²
¹Internal Medicine, University of Cincinnati, Cincinnati, OH, United States; ²HAEA Organization, Honolulu, HI, United States

Background: The objective was to assess HAE patient experience with short term prophylaxis (STP) before surgical/medical procedures (SMP).

Method: An on-line questionnaire was distributed to HAE patients to obtain information about STP and efficacy. The first 250 respondents were included however only data from Type I and II HAE patients (*n* = 219) were analyzed using descriptive statistics.

Results: Demographics of respondents: Average (AVG) age 40 years (range 7–82); Type 1–182(73%), Type 2–37(15%); AVG # of attacks/year 29 mostly extremities and abdomen; 13% previously intubated; 74% with prodromes of which 85% predicted an attack; most frequent triggers (>50%)-emotions (87%), physical stress (79%), trauma (77%), spontaneous (74%), hormones (54%); most frequent SMP triggers-dental (40%), surgery (38%), pregnancy (18%), labor and delivery (8%), endoscopy (5%). Patients receiving prophylaxis: 36% (73/201) Cinryze; 21%(43/201) androgens. Patients receiving OD treatment: 42(21%) Berinert, 17(8%) Kalbitor, 18(9%) Icatibant, 7(4%) fresh frozen plasma. 15%(33/219) used STP: SPT alone used in 19/33 (58%) which was effective in 13/19(68%); SPT+OD(x1) used in 14/30(42%) which was effective in 5/14(36%); Only 1/8 (12.5%) requiring >1 OD treatment responded.

Conclusion: A small percentage of HAE patients reported experience with STP possibly due to fear of precipitating attacks. 42% receiving STP still required OD treat-

Table 1 Effectiveness of short term prophylaxis

PROCEDURE	Total (n)	Dental 4 <i>n</i> , Eff (%)	Surgery 6 <i>n</i> , Eff (%)	Endoscopy 1 <i>n</i> , Eff (%)	Dental+Surg 8 <i>n</i> , Eff (%)	Surg+Endo 1 <i>n</i> , Eff (%)	Dental+Endo 6 <i>n</i> , Eff (%)	Dental+Endo+Surg 7 <i>n</i> , Eff (%)
STP								
STP alone		2 2/2 (100%)	4 2/4 (50%)	..	3 3/6 (50%)	1 1/1 (100%)	5 5/5 (100%)	4 4/6 (67%)
STP+OD(x1)		0	1 1/1 (100%)	1 1/1 (100%)	3 3/3 (100%)	2 1/2 (50%)
STP+OD(x2)		1 0/1 (0%)	1 1/1 (100%)
STP+OD(x3)		1 0/1 (0%)	1 0/1(0%)
STP+OD(x4)		2 0/2 (0%)	..	1 0/1 (0%)	..
OD alone		..	1 0/1 (0%)

ment supporting current recommendations for STP before SMP and having OD treatment readily available for breakthrough attacks.

623

Barriers to the treatment of hereditary angioedema by self-administration: results of a physician survey

Boysen, HB¹; Bouillet, L²; Neri, S³; Hébert, J⁴; Craig, T⁵; Aygören-Pürsün, E⁶; Martinez-Saguer, I⁷; Bethune, C⁸
¹HAEi – International Patient Organization for C1 Inhibitor Deficiencies, Aarhus, Denmark; ²Grenoble University Hospital and National Reference Centre for Angioedema, Grenoble, France; ³University of Catania Policlinico, Catania, Italy; ⁴Centre de Recherche Appliquée en Allergie de Québec, Québec, Canada; ⁵Penn State University, Hershey, PA, United States; ⁶University Hospital Frankfurt, Frankfurt, Germany; ⁷Hämophilie-Zentrum Rhein Main, Mörfelden-Walldorf, Germany; ⁸Derriford Hospital, Plymouth, United Kingdom

Background: Hereditary angioedema (HAE) treatment options include prophylaxis to prevent attacks and emergency treatment to provide rapid relief or prevention of complications during an attack. As attack timing and severity are unpredictable, all patients should have an acute attack management plan. The option of self-administration is offered to patients but uptake varies. Advice on current practice of self-administration and best practice was discussed at an international HAE expert meeting held in Geneva.

Method: A 16-question survey related to HAE treatment and self-administration was sent to 21 centres across Europe, the United States and Canada. Ten centres completed their questionnaires and results were used to stimulate discussion at the international HAE expert meeting to gain an insight into current practice of HAE self-administration, focusing on treatment of acute attacks.

Results: Survey participants identified the main barrier to self-administration as difficulty in injection and infusion skill set (mainly for intravenous treatment). Discussions highlighted that more trained nurses were required, especially in smaller centres. Interestingly, most participants felt that once patients were trained most retained their skills long-term. Eligible patients must be voluntary, adherent, be able to be educated and present with sufficiently frequent attacks to maintain their skills. Although there were no major concerns with offering self-administration, participants did discuss the possibility of infection as a potential risk; however, consensus was that infection rates were no different to those seen in the clinic. All participants of the international HAE expert meeting felt that quality of life was improved with

self-administration. Emphasising the benefits associated with this could help with patient motivation and encourage further patients to adopt self-administration.

Conclusion: Barriers to the treatment of HAE were identified by participants of the international HAE expert meeting and solutions proposed. Participants perceived self-administration to be an advantage to their patients, by improving quality of life and enabling them to manage their disease. The international HAE expert meeting participants believed all patients should be considered for self-administration; however, some patients may need more support than others. Training programmes for medical staff and patients should be implemented to help with disease management.

624

Type-III hereditary angioedema-like syndrome responsive to C1-esterase inhibitor replacement therapy

O'Keefe, A¹; McCusker, C¹; Ben-Shoshan, M¹
¹McGill, Pediatrics – Allergy and Immunology, Montreal, QC, Canada

Background: Hereditary angioedema (HAE) has been classified as a quantitative (type I) or functional (type II) deficiency of C1-esterase inhibitor (C1-INH). More recently, a third type of HAE has been described in patients with normal C1-INH. While C4 levels are characteristically low in HAE type I and II they are within the normal range in patients with type III HAE. Type III HAE is a highly heterogeneous and includes cases related to factor XII mutation as well as cases with an unknown genetic mutation. Further, high estrogen levels are reported to trigger angioedema in HAE type III.

We describe a case of recurrent oropharyngeal angioedema in a 16-year old male with a history of sickle cell disease and thrombocytopenia with no family history of angioedema. His angioedema resulted in severe dyspnea requiring oxygen supplementation. Endoscopy performed during the episode revealed substantial upper airway obstruction.

Results: Emergency treatment of angioedema with C1-INH provided immediate relief, avoiding the placement of a surgical airway. Further evaluation has shown normal levels of C3, C4 and normal levels and function of C1-INH.

Conclusion: Our case exemplifies that regardless of the etiology of HAE, prompt treatment with C1-INH may be an effective and life saving management strategy.

625

The impact of hereditary angioedema on patients' daily life

Gulbahar, O¹; Gökmen, NM¹; Erdogan, AP¹; Erdogdu, D²; Koc, ZP¹; Sin, AZ¹; Ardeniz, O¹; Gurlek, F¹; Buyukozturk, S²; Gelincik, A²; Kokuludag, A¹
¹Internal Medicine Allergy & Immunology, Ege University Medical Faculty, Izmir, Turkey; ²Division of Allergy, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

Background: Hereditary angioedema is characterised by C1-esterase inhibitor deficiency resulting in unpredictable and recurrent subcutaneous edema and mucosal swelling of respiratory and gastrointestinal tracts. We performed this study to investigate the impact of the disease on patients' daily life.

Method: Twenty-nine patients were questioned about the effects of the disease on their life using a 0–5 scale (0: no effect; 5: insupportable).

Results: The majority of patients were female (58.6%). The mean age was 39.10 ± 13.62 years. The mean age at the time of first angioedema symptom was 11.13 ± 6.33 years. Age at the time of diagnosis was 31.44 ± 14.14 . The main triggering factor was stress (69%), followed by trauma (65.5%). Thirty-one percent reported no obvious factor for an attack. In general 3.4% of the patients' defined the disease as insupportable and 13.8% very severe. Severe to insupportable symptoms were present in 55.1% of the patients for abdominal attacks, followed by respiratory (48.2%) and cutaneous (40%) symptoms. Forty-eight percent reported that the disease had negative impacts on their future projects. Thirty-four percent had a relative that died because of this disease, and 6.9% had witnessed to a death of a family member. Only 44.8% of the patients reported that the disease was under control with the medications. Forty-seven percent of the females defined the side effects of the drugs (mainly anabolic steroids) as severe to insupportable.

Conclusion: Hereditary angioedema has serious impacts on patients' lives. Every attempt should be taken in consideration to increase their quality of life.

626

Two village screening of hereditary angioedema on the basis of on index case

Keskin, Ö¹; Özkars, MY¹; Bayram, N²; Küçükosmanoğlu, E¹; Zeyrek, D³; Bayram, H²

¹Pediatric Allergy, University of Gaziantep, G.Antep, Turkey; ²Chest Diseases, University of Gaziantep, G.Antep, Turkey; ³Pediatric Allergy, University of Harran, Ş. Urfa, Turkey

Background: Inherited as an autosomal dominant hereditary angioedema (HAE), 1/10 000–150 000 frequency is observed. Characterised by recurrent episodes of angioedema is a deadly disease. Basically, a protein called C1 inhibitor deficiency or insufficient due to function. Tend to become worse with puberty and after the disease lasts a lifetime. Diagnosis can be an average of 10 years.

Method: Totally 124 person (60 male, 64 female) were screened in two villages on the basis of on index case. The frequency and severity of symptoms were scored from zero to eight. C4 and C1 esterase inhibitor protein (C1-INH) levels were measured in 124 person.

Results: Low C4 levels were found in 42 persons, 82 persons were found healthy. There were 35 patients with C1-INH deficiency. There was a positive correlation between C1-INH and C4 levels ($N = 124$, $P < 0.0001$, $r = 0.81$).

There was a negative correlation between C1-INH levels and symptom score in people who are older than 18 years old ($P = 0.003$, $r = -0.417$, $N = 50$).

Conclusion: It's well known that there's a long term period of delay in the diagnosis of HAE. Our study showed the importance of screening of HAE on the basis of on index case noteworthy, none of the patient with HAE was diagnosed before our screening. Additionally we showed the negative correlation between C1-INH level and symptom scored.

627

Impact of C1 inhibitor self-administration on clinical outcomes in hereditary angioedema patients

Squeglia, V¹; Bova, M¹; Petraroli, A¹; Staiano, R¹; Parente, R²; Marone, G¹; Triggiani, M²; Del Mastro, A³

¹University of Naples 'Federico II', Allergy and Clinical Immunology, Naples, Italy; ²University of Salerno, Division of Allergy and Clinical Immunology, Salerno, Italy; ³University of Naples 'Federico II', Allergy and Clinical Immunology, Naples, Italy

Background: Hereditary angioedema due to C1-inhibitor deficiency (HAE) is a genetic disorder characterised by recurrent attacks of subcutaneous and/or submucosal edema that resolve within 48 to 96 h. Replacement therapy with intravenous plasma-derived C1-inhibitor (pdC1INH) is used to treat acute HAE attacks. New guidelines indicate that home pdC1INH self-administration programs should be offered to patients. We report our experience in the treatment of acute HAE attacks with pdC1INH using home therapy under clinical supervision.

Method: Sixty HAE patients are currently seen in our Divisions. In the last 2 years patients were offered the possibility to attend self-administration courses. Courses were attended by 17 patients and 10 patients' relatives (parents were involved for patients <18 years old). Thirteen of the 17 patients chose to participate in the home therapy program: three patients administered the drug themselves while in the 10 remaining patients the infusion therapy was administered by their relatives.

Results: The number of hospitalisations for pdC1INH administration before home therapy program was 131 on 323 attacks (41%) per year, with an average of 10 infusions per patient. The average time between the onset of attack and infusion of pdC1INH was 3.7 h while the average time to initiation of relief and complete resolution of symptoms was 1.4 h and 15.3 h, respectively. Lost days of work/school were 245 per year with an average of 18.8 for person and 0.8 for attack. After self-administration courses the number of

hospitalisations was 36 on 285 attacks (13%) per year, with an average of 2.8 infusions per patient. The average time between the onset of attack and the infusion was 2.5 h while the average time to initiation of relief and complete symptom resolution was 0.9 and 13.4 h, respectively. The missed days of work/school were 87 per year with an average of 6.7 for person and 0.3 for attack. Only 2 patients experienced mild local side effects at the site of injection.

Conclusion: Home treatment and self-administration offer a good and safe alternative to the hospital treatment by providing a significant reduction in the rate of hospitalisations and in the average loss of work/school per person and attack. Furthermore, self-administration shortens the time between the onset of the attack and the initiation of relief or complete resolution of symptoms.

628

Hereditary angioedema: burden of emergency department visits

Viegas, LP¹; Soares, JB¹; Ferreira, MB^{1,2}; Spínola, AS¹; Barbosa, MP^{1,2}

¹Hospital Santa Maria – CHLN, Lisbon – Portugal, Immunoallergy Department, Lisbon, Portugal; ²Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal

Background: Hereditary angioedema (HAE) is a rare disease caused by C1 inhibitor (C1INH) deficiency leading to recurrent attacks of edema of the skin, gastrointestinal or respiratory tracts, with variable severity. Although many attacks are managed at home by the patients, others lead to Emergency Department (ED) visits.

Aim: Evaluate the burden of our hospital's ED visits of a HAE patients' cohort from Jan/2011 to Dec/2012.

Method: Retrospective study of ED visits registered in ALERTTM electronic database of our hospital. We evaluated the number of ED visits per patient (pt), clinical manifestations, treatment received, duration of

Table 1 Treatment, duration of ED stay and outcome of different HAE attacks

Attack location	Number of visits (Number of patients)	TREATMENT Number of visits with that treatment					Duration of emergency department stay (minutes)	Outcome	
		C1 inhibitor concentrate	Icatibant	Antifibrinolytics	Analgesics/ antiemetics	None		Admission	Discharge
Abdomen	37 (14)	12	14	1	7	3	374/345/136/947	12	25
Pharynx-larynx	9 (8)	5	4	0	0	0	487/407/215/826	4	5
Face	7 (6)	1	4	1	0	1	358/335/47/580	3	4
Extremities	5 (5)	1	1	1	0	2	215/193/31/373	0	5
TOTAL	58 (24)	19	23	3	7	6	377/359/31/947	19 (13)	39 (11)

ED stay and outcome (hospital admission or ED discharge).

Results: Of a total cohort of 97 HAE patients followed in our Department, 24 (24.7%) pts visited the ED for an HAE attack 58 times in the period studied (mean 2.4 visits/2 years/pt, median 1.5; min 1, max 9).

Clinical manifestations were as follows: abdominal – 37; pharyngolaryngeal – 9, face – 7, extremities – 5. Pain scale assessment was available in 30 pts with abdominal attacks: VAS score 0–2: 5 pts (16.7%); 3–5: 14 pts (46.6%); 6–10: 11 pts (36.7%).

Conclusion: Most of our HAE patients didn't have any ED visit in 2 years, reflecting a good overall disease control of our cohort. However 25% of our patients had recurrent ED visits and/or hospital admissions. Most ED visits were due to abdominal attacks, four times more frequent than pharyngo-laryngeal attacks as a reason to seek ED assistance. Early administration of Icatibant or C1INH could imply reduction in ED length of stay and in patients' pain in abdominal attacks. Furthermore, some patients with abdominal attacks did not receive adequate therapy, a fact that underlines the usefulness of home therapy.

629

Practicalities of intravenous C1-inhibitor concentrate self-administration for hereditary angioedema: discussion from an international hereditary angioedema expert meeting

Symons, C¹; Sala-Cunill, A²; Cancian, M³; Rossi, O⁴; Magerl, M⁵; Andritschke, K⁶
¹Derriford Hospital, Plymouth Hospitals NHS Trust, Plymouth, United Kingdom; ²Hospital Universitari Vall d'Hebron, Barcelona, Spain; ³Clinica Medica 1, University of Padua, Padova, Italy; ⁴University of Florence, Florence, Italy; ⁵Charité – Universitätsmedizin Berlin, Berlin, Germany; ⁶HZRM, Mörfelden-Walldorf, Germany

Background: Treatment options for hereditary angioedema (HAE), an autosomal dominant disease, have increased in recent years. Until recently, it was common practice in most countries that patients had to present at hospital for treatment of an attack. However, the self-administration option is increasingly being offered to patients. Information on current practice of self-administration of intravenous (IV) C1-inhibitor (C1-INH), practicalities of administration and initiatives to encourage further use were discussed at an international HAE expert meeting.

Method: To gain insight into current self-administration practice with IV C1-INH in HAE patients, a 16-question survey was sent to 21 centres in Europe, the United States and Canada. Ten centres completed the survey. The results were used to stimu-

late discussion at the international HAE expert meeting with the aim of identifying best/current practice and guidance on how best to advise patients on self-administering IV C1-INH treatment.

Results: International HAE experts discussed practicalities of treating and training patients in the self-administration technique of IV C1-INH. Self-administration was identified as a way to intervene early in acute attacks and give patients control of their own treatment. Five out of 10 centres reported 50–74% of their patients self-administer treatment; 2/10 had 75–89%, 1/10 had 25–49% and 2/10 had 10–24%. Staff members were the main people responsible for training patients (8/12 responses). Participants highlighted that, in addition to training, it was useful to provide guidance on timing of administration and the importance of being fully hydrated. As an attack can happen at any time, implementation of a 24-hour nurse network/helpline (as available for haemophilia patients) was viewed as a useful resource.

Conclusion: The practicalities of self-administration, as discussed at an international HAE expert meeting, highlighted different practices across Europe. All participants agreed the majority of patients were learning the techniques faster than expected. A nurse network/24-hour helpline initiative, as available for haemophilia patients, may be a useful resource for patients to rapidly obtain advice. Training of healthcare providers was noted as an area for improvement especially in smaller centres where there may be few experienced nursing staff. The number of HAE patients being offered self-administration is increasing, and more can be done to encourage uptake.

630

Successful prophylactic treatment with C1-inhibitor-concentrate in a patient suffering from high-frequency hereditary angioedema attacks

Greve, J^{1,2}; Dominas, N¹; Arnolds, J¹; Bas, M³; Hoffmann, T^{1,2}

¹Department of Otorhinolaryngology, University Hospital Essen, University Duisburg-Essen, Essen, Germany; ²Department of Otorhinolaryngology, University Hospital Ulm, Ulm, Germany; ³Department of Otorhinolaryngology, Technische Universität München, Munich, Germany

Background: Hereditary angioedema (HAE) is a rare, autosomal dominant disorder caused by C1-INH deficiency (type I) or qualitative defect (type II). It is characterised by recurrent episodes of edema, particularly in the abdomen, larynx and skin, with a frequency which can range from just a few attacks to >100 attacks per

year. For treating acute attacks, plasma-derived C1-INH concentrates (Berinert[®], Cinryze[®]), a bradykinin B2 receptor antagonist (Firazyr[®]), and a recombinant human C1-INH (Ruconest[®]) are available in Europe. Short-term and long-term prophylactic treatments were conducted with C1-INH (Cinryze[®]) and attenuated androgens (e.g. Danazol, Stanazol).

Method: We report the case of a 66-year-old patient with type I HAE who suffered a high frequency of edema attacks, particular in the head and neck region who was treated prophylactically with androgens for years and now, since 2012, receives a prophylactic therapy with Cinryze[®]. Breakthrough attacks during both prophylactic treatment regimens were treated with Firazyr[®].

Results: Prophylactic therapy with Cinryze[®] resulted in a significant reduction of attacks compared to a therapy with androgens. Particularly acute, life-threatening swelling of the head and neck area could be effectively prevented.

Conclusion: Cinryze[®] proved to be an effective and save drug for the prophylactic treatment in a patient with a high frequency of acute HAE attacks.

631

Icatibant for the treatment of repeated attacks of hereditary angioedema: FAST-3 trial open-label extension study phase

Lumry, W¹; Levy, R²; Li, H³; Farkas, H⁴
¹AARA Research Center, Dallas, TX, United States; ²Family Allergy and Asthma Center, PC, Atlanta, GA, United States; ³Shire HGT Inc., Lexington, MA, United States; ⁴3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary

Background: Hereditary angioedema (HAE) is characterised by recurrent episodes of edema of the skin, gastrointestinal, and/or upper respiratory tract. HAE is caused by deficiency of functional C1-inhibitor and angioedema is mediated by elevated bradykinin levels. The clinical efficacy and safety of icatibant, a bradykinin B2 receptor antagonist, have been established in the phase III For Angioedema Subcutaneous Treatment (FAST)-3 (NCT00912093) study. Here we present data from the controlled and open-label extension (OLE) phase of that trial.

Method: FAST-3 was a randomised, double-blind, placebo-controlled, multicenter study of icatibant for the treatment of adult patients during acute HAE attacks. Patients with moderate to very severe cutaneous and/or abdominal symptoms or mild to moderate laryngeal symptoms were randomised to a single subcutaneous injection of icatibant 30 mg or placebo at the time of their first HAE attack. For subsequent attacks, patients could receive open-

label icatibant regardless of initial randomisation. The primary endpoint was the time from icatibant treatment to onset of patient-reported symptom relief (skin swelling, skin pain, and abdominal pain), defined as a 50% reduction from the pre-treatment score in the composite visual analog scale (VAS) score. Time to onset symptom relief using VAS score for a single primary symptom and safety were secondary endpoints. Analyses were performed on the first 5 icatibant-treated attacks across controlled and OLE phases.

Results: Four hundred and eighty-nine attacks overall and 435 attacks in the OLE, were treated with icatibant. The majority of OLE attacks (95%; $n = 415/435$) were treated with 1 icatibant injection. Across the first 5 icatibant-treated attacks (281/489 attacks), the median time to onset of symptom relief (composite VAS score) was between 1.9 and 2.1 h, and median time to onset of primary single symptom relief was between 1.5 and 2.0 h. Serious adverse events (SAEs) occurred in 1.1–5.4% of patients with two patients reported as having a possible drug-related SAE. Injection site reactions were mostly mild to moderate and transient; no deaths were reported.

Conclusion: These data demonstrated that icatibant was efficacious over repeated treatment of multiple HAE attacks. The majority of attacks required only 1 injection, and sustained efficacy was observed across the first 5 icatibant treated attacks. The safety profile for icatibant was consistent with other icatibant phase 3 trials.

632

A cross-sectional questionnaire survey to assess physicians' approach to short-term prophylaxis in hereditary angioedema patients

Bernstein, JA¹; Singh, U²; Wilmont, J³

¹Internal Medicine, University of Cincinnati, Cincinnati, OH, United States; ²University of Cincinnati, Cincinnati, OH, United States; ³Hereditary Angioedema Association, Honolulu, HI, United States

Background: To assess the physician's treatment approach for HAE patients

undergoing elective invasive surgical/medical procedures (SMP).

Method: A retrospective cross sectional on-line survey was administered to physicians to assess use STP for Type I or II HAE patients with history (Hx) or regardless of history (\pm Hx) of trauma-induced swelling during minimally invasive SMP (MISMP; dental work, endoscopy), invasive (ISMP; laparoscopy, surgery, C-section) and normal vaginal deliveries (NVD). STP and on-demand (OD) medications included pdC1INH (Berinert or Cinryze), Ecallantide, Icatibant, fresh frozen plasma (FFP), anabolic steroids (AS) and anti-fibrinolytic agents (AFA). Questionnaire responses were analyzed using descriptive statistics.

Results: Thirty-seven respondents treated 177 HAE patients requiring an ER visit in the past year; 46 were hospitalised and 12 intubated. 32/37(86%) routinely prescribed SPT and/or OD therapy for MISMP or ISMP procedures. For SPT, 14 used Cinryze, 6 AS and 1 AFA; for OD, 32 used Icatibant, 5 Berinert, 4 FFP, 2 Ecallantide. For OD-MISMP, OD-ISMP and OD-NVD uses, Icatibant, Berinert, FFP, Ecallantide were prescribed from greatest to least frequency. For all STP scenarios (MISMP, ISMP, NVD Hx or \pm Hx) Cinryze was most commonly prescribed.

*OD [\times] = number of additional OD treatments.

Conclusion: SPT of HAE patients undergoing MISMP or ISMP procedures was most effective in patients with Hx of trauma induced swelling. The best outcome was SPT with OD therapy readily available for a breakthrough attack.

633

Icatibant effectiveness and utilisation in patients with hereditary angioedema: results from the Czech Republic database

Křmčová, I¹; Skoupá, J²; Kuklínek, P³; Zachová, R⁴; Hanžlíková, J⁵

¹University Hospital Hradec Králové, Institute of Clinical Immunology and Allergology, Hradec Králové, Czech Republic; ²Medical Data Center, Charles University, Prague, Czech Republic; ³Department of Clinical Immunology and Allergology, University Hospital U Sváté Anny, Brno, Czech Republic; ⁴Department of Immunology, Charles University and University Hospital Motol, Prague, Czech Republic; ⁵Department of Immunology and Allergology, University Hospital, Plzeň, Czech Republic

Background: Hereditary angioedema (HAE) results in elevated plasma levels of bradykinin, a key mediator of disease symptoms. Icatibant is a selective bradykinin B2 receptor antagonist which is approved for HAE type I and type II use in Europe and currently has temporary insurance reimbursement in specified HAE centers in the Czech Republic. The Icatibant Database: Czech Republic is a mandatory registry for all patients prescribed icatibant. To assess the effectiveness and utilisation of icatibant, a retrospective analysis of this database was conducted.

Method: Demographics, HAE history, current therapy, frequency and location of attacks during previous 12 months, and healthcare utilisation were assessed at the baseline visit (V0). For all subsequent visits (V1+), number of attacks with and without icatibant utilisation, time to recovery (defined as time to perceived improvement after injection as stated by the patient), rate of self-injection, and healthcare utilisation were recorded.

Results: From March 18, 2011 to September 30, 2012, 113 patients were enrolled (female: 42%; mean age: 40 years; prophylactic treatment use (mainly danazol): 77%) with a mean time assessment of 465 days. The average number of HAE attacks within the previous 12 months was 6.20 per patient. From the time of database entry, 750 attacks were recorded; 232 attacks were treated with 239 injections of icatibant (mean injection rate: 1.03 injections per attack). The average time to

Table 1 Physician Use of STP

Regimen	MISMP			ISMP			NVD	
	#Physicians n%	Effective	Regimen	#Physicians n%	Effective	Regimen	#Physicians n%	Effective
STP(Hx)	5	3(3/5) 60%	STP(Hx)	3	2(2/3) 67%	STP(Hx)	1	1(1/1) 100%
STP(Hx) + OD[\times 1]	2	2(2/2) 100%	STP(Hx) + OD[\times 1]	1	1(1/1) 100%	STP(Hx) + OD[\times 1]	0	
STP(\pm Hx)	8	3(3/8) 38%	STP(\pm Hx)	12	3(3/12) 25%	STP(\pm Hx)	5	2(2/5) 40%
STP(\pm Hx) + OD[\times 1]	5	4(4/5) 80%	STP(\pm Hx) + OD[\times 1]	9	8(8/9) 89%	STP(\pm Hx) + OD[\times 1]	3	2(2/3) 67%
STP(\pm Hx) + OD[\times 2]	1	1(1/1) 100%	STP(\pm Hx) + OD[\times 2]	1	1(1/1) 100%	STP(\pm Hx) + OD[\times 2]	1	1(1/1) 100%
OD[\times 1] only	2	2(2/2) 100%	OD[\times 1] only	0		OD[\times 1] only	0	

recovery was 2 h and by V4, 100% of patients self-injected. The percentage of patients requiring hospitalisation, emergency medical service, and rapid emergency medical service was lower after icatibant prescription than in the previous 12 months. A similar trend was seen for percentage of attacks requiring healthcare utilisation. The greatest difference was seen in patients requiring hospitalisation (0.93% of patients with icatibant vs 2.43% of patients in previous 12 months) and attacks requiring hospitalisation (0.93% of attacks in patients with icatibant vs 2.85% of attacks in previous 12 months).

Conclusion: This database represents a patient population with HAE in the Czech Republic. Results from this retrospective analysis show that icatibant was used in approximately 30% of all attacks, and when icatibant was administered there was a decrease in overall healthcare utilisation. In addition, patients were readily able to learn to self-administer icatibant.

634

Comparison of costs for on-demand treatment of hereditary angioedema symptoms in the United Kingdom

Jolles, S¹; Machnig, T²; Zbrozek, A³

¹Department of Immunology, University Hospital of Wales, Cardiff, United Kingdom; ²CSL Behring GmbH, Marburg, Germany; ³CSL Behring LLC, King of Prussia, PA, United States

Background: Treatment options approved in the United Kingdom (UK) for on-demand use for type I or II attacks of hereditary angioedema (HAE) provide similar efficacy. They differ, however, by the doses administered per patient and price per administration, weighted in part by the need for re-dosing.

Method: A cost minimisation analysis was used to compare the differences in processes required to achieve resolution of HAE attacks with four drugs approved in the UK: Berinert[®] (CSL Behring, Germany), Cinryze[®] (Sanquin, The Netherlands), Firazyr[®] (Shire, United States), and

Ruconest[®] (Pharming Group N.V, The Netherlands). The differential costs were compared indirectly using the official NHS costs according to the All Wales Medicines Strategy Group criteria. The results were weighted by re-dosing frequency estimates.

Results: Based on clinical trial data, frequencies for re-dosing with on-demand treatment were estimated as 1% for Berinert, 30.9% for Cinryze, 7% for Firazyr, and 10% for Ruconest. Since Berinert is dosed based on weight, its dosing in this analysis ranged from 2 to 4 vials for patients weighing 50 to 100 kg. An estimated cost of care in hospital of £13 was applied to Ruconest. Per attack, Berinert was the least costly therapy when dosed with either 2 or 3 vials. For patients with higher body weight needing 4 vials of Berinert, savings were maintained vs Cinryze and Ruconest.

Conclusion: Berinert for on-demand treatment of HAE in the UK appeared to be the least costly therapy for a large proportion of patients.

Poster Session 14

New insights into urticaria

636

Concurrent chronic idiopathic urticaria and autoimmune hepatitis

Peña Arellano, M¹; Flores Martín, IM¹; Martín Furio, RM¹; Berenguer Trives, E¹; Exposito Barroso, F²; Miras Bruno, JA²

¹Hospital Vega Baja, San Bartolome, Spain; ²Hospital Virgen de la Arrixaca, El Palmar, Spain

Background: Chronic urticaria is defined as appearance of 24 h of duration wheals for at least 6 weeks. It is a common skin disease without clear etiology in most cases. The presence of serum IgG autoantibodies targeting IgE or the IgE receptor in approximately 40% of chronic idiopathic urticaria cases supports the theory of an autoimmune basis for the disease.

Method: We present a 34 years old woman who suffers chronic urticarial, since 2 years. She had been taken treatments with loratadine, rupatadine and several oral steroids cycles without clinic control. Personal history: she has rhinitis in the spring and autumn, without other chronic disease or treatments associated. She didn't drink alcohol

Results: Prick test: positive to olive and Chenopodiaceae pollen

Blood test: Hemogram and coagulation: normal erythrocyte sedimentation rate: normal ALT, AST 114 U/l (0–37), ALT 260 U/l (0–42) Alkaline phosphatase 107 U/l (40–136), total bilirubin: normal, LDH 160 U/l (100–190), GGT 220 U/l (5–85). Iron and ferritin: normal. Afetoprotein: normal Ig G: 2040 mg/dl (700–1600). α 1 antitripsin and ceruloplasmin normal: copper levels in serum and urine: normal: thyroid hormones: T3, T4, TSH: normal markers of viral hepatitis: negative: Antinuclear antibodies (ANAs) positive 1/160, Smooth muscle antibodies (SMA) positive 1/640, anti Ro-52 positive. AN-CAs, AMA-M2 3E(BPO), Sp100, PML, gp210, LKM-1, LC1, SLA/LP, antibodies antiactin: negative

Liver ecography: light hepatic steatosis

Liver biopsy: lymphoplasmacytic infiltration with eosinophils in the portal tracts. Occasionally limiting erosion. The lobule: histiocytic nodules, occasional apoptotic hepatocytes and foci with sinusoidal lymphocytosis

Autoimmune Hepatitis Score: 15 probable autoimmune hepatitis. The patient is

assessed by digestive specialists who diagnosed her: Autoimmune Hepatitis. After autoimmune hepatitis diagnosis the patient was treated with prednisone and azathioprine. After that, the hives disappeared and transaminase levels were normalised, even after decreasing prednisone doses.

Conclusion: The association of urticaria and autoimmune disorders such as thyroid disease, is well known, but there are few cases reported with concomitant idiopathic urticaria and autoimmune hepatitis. We report a patient with chronic urticaria clinic associated with autoimmune hepatitis, with well response from both entities after starting treatment with prednisone and azathioprine.

637

Delayed urticaria induced by antihistamines

Ruiz León, B¹; Cándón Morillo, R¹; Burgos Montero, A¹; Moreno Mata, E¹; González Sánchez, LA¹; Araque Arroyo, P²

¹Allergy Department, La Mancha Centro Hospital, Alcazar de San Juan, Ciudad Real, Spain; ²Pharmacy Department, La Mancha Centro Hospital, Alcazar de San Juan, Ciudad Real, Spain

Background: H1-Antihistamines are widely used drugs for allergic diseases. Antihistamines are the most commonly used drugs in the treatment of urticaria, they can rarely cause adverse cutaneous reaction, however a few cases Antihistamines-induced urticaria have been reported.

We report a case of a patient with episodes of urticaria induced by different classes of Antihistamines.

Method: The patient was a 52 year old woman with a history of AAS and Pirazolona allergic, and she presented since 3 month ago, wheal and flares with itching on her forearms and after 4–5 h of taking one tablet of levocetirizine 5 mg, she developed itching, wheal and flares urticaria extended to the entire body surface.

Skin prick test were performed with a series of commercially available common inhalant, latex and Anisakis. To evaluate hypersensitivity to H1 antihistamines, we carried out skin prick tests, patch test and a single-blind, placebo-controlled oral challenges with antihistamines agents.

Results: His physical examination was normal. The complete blood count, chemistry, complement, thyroid hormones were normal. Subclinical sensitisation to Anisakis simplex, salsola Kali, olea europea and mites.

- Skin prick test and patch test with Levocetirizine, Cetirizine, Dexchlorpheniramine, Loratadine, Hidroxyzine, Rupatadine, fexofenadina, Ebastine, Bilastine were negative.
- Four-five hours after oral challenges with Levocetirizine, Cetirizine, Dexchlorpheniramine, Loratadine, Hidroxyzine, Bilastine; she developed an generalised urticaria reaction. The patient tolerated an oral challenge with Ranitidine.

Consequently she was diagnosed with urticaria induced by multiple H1-antihistamines.

Conclusion: We presented a patient who developed delayed urticaria after the intake of different families of antihistamines. Adverse drug reactions to histamine H1 antagonists are rare, cutaneous adverse reactions to orally administered antihistamines usually appear within 4–12 h. The mechanism of AH induced urticaria has not yet been elucidated; the reactions have been classified as type I or IV on the basis of the clinical history and time between intake and reaction. We have established the diagnosis by oral challenge.

638

Chronic urticaria: a three year review

Moreira, AS¹; Rosmaninho, I¹; Guilherme, A¹; Silva, JM¹

¹Centro Hospitalar de Vila Nova de Gaia e Espinho, Imunoalergologia, Vila Nova de Gaia, Portugal

Background: Chronic urticaria (CU) is an increasingly prevalent and debilitating disease which requires effective treatment. The aim of our study was to characterise the patients with CU followed in the cutaneous unit of our department, in the last 3 years.

Method: The following data were retrospectively collected from the CU patients evaluated between January 2010 and December 2012: gender, age, comorbidities, duration of disease at first visit, eliciting factors, urticaria types (UT) and response to treatment.

Results: We studied 165 patients, 73% of which were females. Median age was 39 years (3–88). The main comorbidities found were: allergic disease (27%), depression (10%) and thyroid disease (8%). The disease duration at first visit was ≤ 1 year in 54% cases, 1–5 years in 31%, 5–10 years in 7% and ≥ 10 years in 8%. The most prevalent eliciting factors were pressure (26%), stress (17%) and body temperature increase (16%). 42% of patients had widespread urticaria. The Urticaria Score Activity was 3–4 in 52% of the cases. The most prevalent UT were chronic spontaneous urticaria (CSU) 49%, physical urticaria 44% (dermographic urticaria (DU) 33%, cold urticaria 6%, delayed pressure urticaria (DPU) 3%, solar urticaria 3%) and cholinergic urticaria 16%. Different UT were found simultaneously in 10% of the cases. All UT were more prevalent in females. 69% of patients responded to treatment with antihistamines (AH) in standard dosage, 20% to fourfold AH up-dosing, 8% to leucotriene antagonist+AH, 2% to association of 2 AH and one patient to omalizumab. Angioedema was present in 34% cases (80% of the cases of DPU, 41% of CSU, 33% of cholinergic urticaria, 24% of DU, 20% of solar urticaria and 11% of cold urticaria).

Conclusion: Gender and median age of the patients studied were similar to those referred in literature. The majority of patients had their first visit when disease duration was ≤ 1 year. The most prevalent UT was CSU. Angioedema was more prevalent in DPU. Standard dosage of AH was an effective treatment for the majority of patients.

639

Treatment with off-label omalizumab in chronic idiopathic histaminergic urticaria – angioedema resistant to high doses of antihistamines

Rijo Calderón, Y¹; Palao, P¹; Prior, N¹; Fiandor, A¹; López-Serrano, MC¹; Olalde, S¹; Quirce, S¹; Caballero, T¹

¹Allergy Department, Hospital La Paz Institute for Health Research (IdiPaz), Madrid, Spain

Background: Idiopathic chronic spontaneous histaminergic urticaria – angioedema (ICSHU-AE) is frequently unresponsive to treatment with high doses of antihistamines, needing long therapies with oral corticosteroids or other immunosuppressants. Omalizumab has been reported to be beneficial in the aforementioned patients.

Method: A retrospective descriptive analysis was performed in patients diagnosed of ICSHU-AE resistant to high doses antihistamines, in whom off-label omalizumab

had been prescribed during 2011–2012 in our department.

Results: Twelve patients were included (8 female, 4 male). The mean age at beginning of symptoms was 35.5 year (M: 34, F: 39). Six presented urticaria-angioedema (all female), 4 angioedema (M: 3, F: 1) and 2 urticaria (M: 1, F: 1). Before the treatment with omalizumab all the patients had been treated with antihistamines plus corticosteroids and four patients also with dapson.

The initial dose of omalizumab was 150 mg in five patients, 225 mg in 2 patients and 300 mg in five patients. The initial interval was 4 weeks for 11 patients and 2 weeks for one patient.

All the patients presented improvement after the first dose, including two patients diagnosed with urticaria-angioedema vasculitis. Five patients were asymptomatic after first dose and seven continued with mild symptoms. Six patients suspended the treatment previous to omalizumab just after the first dose, two patients after the second one. Three of them continued with antihistamines, one continued with oral corticoids up to the seventh dose and other was able to stop dapson and to decrease oral corticosteroid.

Initial omalizumab dose could not be lowered in any patient, but the interval between doses could be increased in three patients (6 weeks in 1 pt; 5 weeks in 2 patients). Omalizumab was withdrawn because of improvement after the sixth dose in one patient.

Mild side effects were seen in seven patients, being the most frequent drowsiness (7 patients), followed by digestive, cutaneous symptoms, and loss of weight (5 patients). Omalizumab was not stopped in any patient because of them.

Serum total IgE geometric mean was 101.2 kU/l before treatment and 178.5 post-treatment. The average number of eosinophils was 231.9 cells/mm³ prior to treatment and 197.2 post-treatment.

Conclusion: Omalizumab was shown to be effective and safe in patients with ICSHU-AE unresponsive to other treatments.

640

Acute urticaria in the emergency department: assessment of 565 cases

Pitchon, R¹; Reis, DP²; Reis, AP³; Chuster, A³; Montovani, R⁴; Cunha, MP⁴; Magno, A⁴; Alves, V⁴; Fausto, P⁴

¹Allergy Pediatrician, Hospital Mater Dei, Belo Horizonte, Brazil; ²Pediatrics, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; ³Pediatrics, Universidade Ciências Médicas, Belo Horizonte, Brazil; ⁴Pediatrics, Hospital Mater Dei, Belo Horizonte, Brazil

Background: Assessment of patients admitted with acute urticaria in the Pediatric Emergency Department.

Methods: Uncontrolled study of 565 children and adolescents aged from 0 to 15 years, from January 2012 to December 2012, with acute urticaria (ICD L50).

Results: In this period, a total of 64 203 cases were admitted in the Emergency Department, of these 565 (0.88%) with acute urticaria. The incidence was higher in males (56%) and in children aged between 1 and 3 years old, which corresponded to 45.5% of the total of cases diagnosed with acute urticaria. Hospital medication was prescribed to 33.27% of the patients. Sixty one cases (10.79%) were diagnosed with associated diseases, and the most frequent were infectious diseases in 45 cases (75%). The most common infectious diseases were otitis, acute rhinofaryngitis, acute sinusitis, influenza and acute tonsillitis (see Table I). In a period of less than 72 h after the hospital discharge, 19 patients (3.36%) were readmitted. Five patients were hospitalised, one case associated with bacterial pneumonia and another with cellulitis.

Conclusion: Performance measurement of care quality allows the comparison of data between institutions and the creation of incentives for improving care quality standards. After the results, the staff did a protocol for the attendance of acute urticaria and a discharge standard prescription for the patient and his family.

641

Is presence of MEFV gene mutation a protective factor for chronic idiopathic urticaria?

Sayin, S¹; Taskin, G¹; Koc, B¹; Yesillik, S²; Arik, D³; Demirel, F²; Arslan, E¹; Tunca, Y³; Sener, O²; Saglam, K¹

¹Department of Internal Medicine, Gulhane Military Medical School, Ankara, Turkey; ²Department of Internal Medicine, Division of Immunology and Allergic Diseases, Gulhane Military Medical School, Ankara, Turkey; ³Department of Medical Genetics, Gulhane Military Medical School, Ankara, Turkey

Introduction: The structural changes of the pyrene molecule due to mutations in the MEFV gene, cause increased leukocyte migration and a prolonged inflammatory response to stimuli. Several studies had shown the relationships between the mutations in the MEFV gene and Behçet's Disease, Rheumatoid Arthritis, Inflammatory Bowel Disease. Because of unknown etiology, we investigated the relationship between MEFV gene mutation and chronic idiopathic urticaria (CIU).

Method: We included 42 patients who were diagnosed as CIU. We compared the patients with the control group for mutation frequency. The patients with MEFV gene mutation negative were also com-

pared with MEFV gene mutation positive patients.

Results: We determined heterozygous MEFV gene mutations at six patients and its frequency was 9.5%. The frequency of carriage of MEFV gene mutations in healthy individuals in Turkish society was 20%. There was no statistically significant difference between the healthy carrier and CIU patients for the frequency of MEFV gene mutations. There was also no significant difference between the patients with MEFV gene mutation negative and MEFV gene mutation positive for age at diagnosis, disease duration, eosinophil count, sedimentation time, and the incidence of urticaria of first-degree relatives.

Conclusion: Despite the detection of MEFV gene mutation in CIU patients was lower than healthy society carriers, there was no statistically significant difference. In addition, the MEFV gene mutation in patients with CIU was not contributed to familial inheritance of the disease, disease duration and severity.

642

Baseline characteristics of patients with refractory chronic idiopathic/spontaneous urticaria enrolled in two phase III, randomised, placebo-controlled trials of omalizumab

Rosén, K¹; Hsieh, H-J¹; Bradley, MS¹; Georgiou, P²; Casale, T³; Maurer, M⁴

¹Genentech, Inc., South San Francisco, CA, United States; ²Novartis Pharmaceuticals UK Limited, Horsham, United Kingdom; ³Creighton University Medical Center, Omaha, NE, United States;

⁴Department of Dermatology and Allergy, Charité-Universitätsmedizin, Berlin, Germany

Background: The characteristics of patients with refractory chronic idiopathic/spontaneous urticaria (CIU/CSU) have been minimally reported to date. Here we describe the baseline characteristics of the study populations from two Phase III trials (Asteria-I and Asteria-II) of omalizumab, an anti-IgE antibody in development for the treatment of patients with CIU/CSU who remain symptomatic despite H₁-antihistamine treatment.

Method: Asteria-I and Asteria-II were global, multicentre, randomised, double-blind, dose-ranging, placebo-controlled studies evaluating the efficacy and safety of omalizumab in patients aged 12–75 years with CIU/CSU (for ≥6 months) who remained symptomatic on approved doses of H₁-antihistamines. Patients were randomised 1:1:1:1 to receive six (Asteria-I) or three (Asteria-II), 4-weekly subcutaneous doses of omalizumab (75, 150 or 300 mg) or placebo over 24 and 12 weeks respectively. The primary end point was Week 12

change from baseline in weekly itch severity score for both studies.

Results: A total of 640 patients (Asteria-I: *n* = 318; Asteria-II: *n* = 322) were randomised and received treatment. Baseline demographic data are shown in the table. In both studies, most patients were female and white with a high body mass index. The median duration of CIU/CSU was >3 years. Angioedema was present in >40% patients at baseline. Baseline characteristics were generally consistent across the treatment groups and both studies. Mean urticaria and itch scores indicated a high degree of disease activity.

Conclusion: In these two studies, the majority of patients with CIU/CSU who remain symptomatic despite H₁-antihistamine treatment were aged ~40 years and female. A high incidence of angioedema was seen. Despite H₁-antihistamines, these patients experienced moderate-to-severe symptoms. The patient characteristics described in these two studies mirror the characteristics of the CIU/CSU patient population reported in the literature and seen in clinical practice.

Baseline characteristic	Asteria-I (<i>n</i> = 318)	Asteria-II (<i>n</i> = 322)
Mean (SD) age, years	41.2 (14.5)	42.5 (13.7)
Mean (SD) weight, kg	82.2 (21.0)	82.4 (21.9)
Mean (SD) body mass index	29.3 (6.8)	29.8 (7.3)
Female, <i>n</i> (%)	231 (72.6)	244 (75.8)
Caucasian white, <i>n</i> (%)	263 (82.7)	272 (84.5)
Median (range) duration of CIU/CSU, years	3.7 (0.5–50.5)	3.3 (0.5–66.4)
Mean (SD) weekly urticaria activity score (UAS7)	31.1 (6.6)	30.7 (6.8)
Mean (SD) weekly itch severity score	14.3 (3.5)	14.0 (3.7)
Presence of angioedema, <i>n</i> (%)	151 (47.5)	131 (40.7)

643

A retrospective multicenter surveillance of anaphylaxis in Korea

Jang, G-C¹; Ye, Y-M²; Kim, MK³; Kang, H-R⁴; Kim, T-B⁵; Sohn, S-W⁶; Koh, Y-I⁷; Park, H-K⁸; Kim, C-W⁹; Jee, Y-K¹⁰; Hur, G-Y¹¹; Kim, J-H¹²; Kim, S-H¹³; Choi, G-S¹⁴; Lee, S-K¹⁵; Park, H-S²; KAAACI Work Group on Urticaria/Angioedema/Anaphylaxis

¹NHIC Ilsan Hospital, Goyang, Korea; ²Ajou University School of Medicine, Suwon, Korea; ³Chungbuk National University, Cheongju, Korea; ⁴Seoul National University College of Medicine, Seoul, Korea; ⁵College of Medicine, University of Ulsan, Seoul, Korea; ⁶Dongguk University Ilsan Hospital, Goyang, Korea; ⁷Chonnam National University Medical School, Gwangju, Korea; ⁸Pusan National University School of Medicine, Pusan, Korea; ⁹Inha University College of Medicine, Incheon, Korea; ¹⁰Dankook University College of Medicine, Cheonan, Korea; ¹¹College of Medicine, Korea University, Seoul, Korea; ¹²Hallym University College of Medicine, Anyang, Korea; ¹³Hanyang University College of Medicine, Seoul, Korea; ¹⁴Kosin University College of Medicine, Pusan, Korea; ¹⁵College of Medicine, Dong-A University, Pusan, Korea

Background: Little is known about the characteristics of anaphylaxis in Korea. We evaluate the incidence of anaphylaxis and the clinical features of patients with anaphylaxis in Korean secondary and tertiary care hospitals.

Method: We performed a retrospective review of 1776 adult patients of anaphylaxis (ICD-10 code: T78.0, T78.2, T80.5, T88.6, T63.4) with same eCRF, including inpatients, outpatients, and emergency department visitors, in 15 Korean secondary and tertiary care hospitals between January 1, 2007, and Dec. 31, 2011.

Results: Among 1776 patients, 792 (44.6%) had past history of allergic diseases and 16.8% had family history of allergic diseases. Mean age was 46.0 ± 16.0 (16–89) y. The causes of anaphylaxes were drug (46.6%), food (24.2%), insect stings (16.6%), exercise-induced (5.9%), idiopathic (5.3%), and others (1.4%). Radiocontrast media and buckwheat were the leading causes of drug and food anaphylaxis, respectively. The organs most frequently involved in the anaphylaxis were cutaneous (84.0%), cardiovascular (55.4%), respiratory (53.9%), general (21.0%), and gastrointestinal (18.5%). The rate of bee venom induced anaphylaxis was relatively high in northern area than seoul metropolitan area. The rate of food induced anaphylaxis was relatively high in winter, and bee venom induced anaphylaxis was relatively high in summer than the other seasons. The cases of annual anaphylaxis had increasing pattern: 224cases (2007), 328cases (2008), 334cases (2009), 390cases (2010), and 493cases (2011).

Conclusion: The incidence, and clinical features of Korean patients with anaphylaxis were similar to rates for patients from other countries, despite some differences in causative agents, regional distribution, and seasonal variation.

644

Clinical features of cold urticaria patients: a nine-year retrospective analysisDemirel, F¹; Gulec, M¹; Yesilcik, S¹; Baysan, A¹; Kartal, O¹; Musabak, U¹; Sener, O¹¹Department of Internal Medicine, Division of Immunology and Allergic Diseases, Gulhane Military Medical School, Ankara, Turkey

Background: Cold urticaria (CU) is a subtype of physical urticarias which is characterised by urticaria and/or angioedema after cold exposure. We present demographical and clinical features of subjects with CU followed by our clinic.

Methods: Detailed medical histories, laboratory tests, cold stimulation test (CST) and atopy test results were reviewed retrospectively.

Results: Seventeen patients (8 male and 9 female) with CU were reviewed. The median admission age was 38 (min: 20, max: 68) and the median duration of symptoms were 3 (min: 1, max: 30) years. While 5 patients (29.4%) with CU had the lesions on all body, 4 patients (23.5%) had on the extremities and faces; 2 patients (11.8%) had on faces and 6 patients (35.3%) had on the extremities only. Seven patients (41.2) were suffering from CU during winter, however 10 patients' (% 58.8) lesions were active all year long. All laboratory tests (complete blood count, erythrocyte sedimentation rate, C-reactive protein, anti-nuclear antibody, rheumatoid factor, antistreptolysin-O, cold agglutinins, cryoglobulins, complement C4 and C3, syphilis, hepatitis B and C and HIV serologies, thyroid hormones, and anti-thyroid peroxidase) were within normal limits. Prick tests with inhalant allergens revealed that 1 patient had pollen and cockroach allergy, 1 patient had pollen and latex allergy and 1 patient had only pollen allergy. CST was positive in 9 patients (52.9%) and negative in eight patients (47.1%).

Conclusion: CU is an important chronic disorder and the pathogenesis is still unknown exactly. Although CST is a useful test for diagnosing the CU, it may not be positive for all the patients. Detailed personal medical histories still have a great importance.

Keywords: Cold urticaria, cold stimulation test

646

Chemokines and biomarkers of endothelial dysfunction in chronic urticariaPuxeddu, I¹; Panza, F¹; Bartaloni, D¹; Pratesi, F¹; Rocchi, V¹; Del Corso, I¹; Migliorini, P¹¹Pisa University, Pisa, Italy

Background: Chronic Urticaria (CU) is a common and disabling disease characterised by recurrent itchy wheals with or without angioedema for more than 6 weeks. These symptoms are the consequence of skin mast cells degranulation with release of histamine and other vasoactive mediators. The aim of our study is to investigate the potential involvement of chemotactic mediators (CCL5/RANTES and CXCL8/IL-8) and markers of endothelial dysfunction such as soluble VCAM-1 (sVCAM-1) and soluble ICAM-1 (sICAM-1) in the pathogenesis of CU, and their relation with disease activity.

Method: We measured the levels of CCL5/RANTES, CXCL8/IL-8, sVCAM-1 and sICAM-1 in the sera of 87 patients with CU and 61 sex- and age-matched normal healthy subjects (NHS), using ELISA assays. According to the results of the autologous serum skin tests (ASST), CU patients were classified in the ASST-positive and ASST-negative subgroups.

Results: We detected significantly higher concentration of CCL5/RANTES ($P < 0.0001$) but not of CXCL8/IL-8 in the sera of CU patients compared to NHS. The serum levels of sICAM-1 and sVCAM-1 were significantly increased in the CU patients compared to the control subjects ($P = 0.0121$ and $P = 0.0043$, respectively). No differences in either chemokines and adhesion molecules levels was detected in the ASST-positive and ASST-negative subgroups. Positive correlation has been found between sICAM-1 and sVCAM-1 but not between them and CCL5/RANTES.

Conclusion: Our study suggests the potential involvement of the C-C chemokine CCL5/RANTES and of the biomarkers of endothelial dysfunction sICAM-1 and sVCAM-1 in the pathogenesis of CU.

647

Omalizumab: an effective and rapidly acting therapy in difficult-to-treat chronic urticariaOhanyan, T¹; Metz, M¹; Church, M¹; Maurer, M¹¹Department of Dermatology and Allergy, Allergie-Centrum-Charité, Charité - Universitätsmedizin Berlin, Berlin, Germany

Background: Omalizumab (anti-IgE) therapy has been demonstrated to be effective

and safe in chronic urticaria (CU) in placebo-controlled clinical trials but practical clinical data are scarce. This report details the use of omalizumab in difficult-to-treat CU patients in routine clinical practice.

Method: We assessed responder rates, optimal dosage, response to up-/down dosing, time to relief of symptoms, rates of return and time to return of symptoms after administration of omalizumab and safety in 51 CU patients, 20 with chronic spontaneous urticaria (CSU) alone, 21 with different forms of inducible urticaria and 10 with both, CSU and inducible urticaria attending the specialist urticaria clinic at our department.

Results: Omalizumab treatment led to complete responses in 83% of CSU and 70% of inducible urticaria patients. With a starting dose of 150 mg omalizumab every 4 weeks, only 2/15 CSU patients and 7/17 inducible urticaria patients who went on to show complete responses required up dosing to achieve this. In CSU, 57% of complete responses occurred within week one, most on day one after the first treatment. Relapse times were between 2 and 8 weeks in all but six patients, where they were longer than 4 months. Omalizumab was safe. Efficacy was not correlated to baseline IgE levels. Omalizumab did not inhibit codeine- or histamine induced wheal and flare responses.

Conclusion: Our clinical experience from more than 1250 injections in 51 patients over 4 years clearly indicates that omalizumab is a rapidly acting, highly effective and safe drug in patients with CSU and inducible urticaria. This supports the recommendation of the current EAACI/GA2LEN/EDF/WAO guideline for the management of urticaria to use omalizumab as a third line treatment in difficult to treat urticaria patients.

648

Sulfite-dependent exercise urticariaDe Luque Piñana, V¹; Egúez Hidalgo, MF¹; Segura Sanchez, C¹; Botello Borrego, MD¹; Cabeza Rodriguez, N¹; Guardia Martinez, P¹¹Allergy and Clinical Immunology Department, Virgen Macarena University Hospital, Seville, Spain

Background: Within the exercise-induced anaphylaxis syndrome two well differentiated clinical forms are included: systemic cholinergic urticaria and exercise induced anaphylaxis in the strict sense which can be shown by a classic form or a variant form, more uncommon and with manifestations similar to cholinergic urticaria. Postprandial or food-dependent exercise-induced anaphylaxis is a frequently identified subtype of these last cases.

Method: A 38 year old woman with history of diabetes mellitus who consults by wheals predominantly on the scalp, arms and face and otic tamponade, with a frequency of four episodes per year, specifying emergency assistance and treatment with corticosteroids and antihistamines. Refers not apparent cause, except when eating away from home or celebrations like weddings, coinciding with the intake of highly processed foods, spicy, rich in preservatives or precooked. She tolerates all foods and medicaments.

In the latest revision refers wheals after ingestion of beef burger precooked followed by physical exercise.

Results: Basic blood analysis, thyroid profile, complement, total IgE, specific IgE parasites, and tryptase, all within normal limits. Skin prick tests to aeroallergens, food, shrimp, clams, mussels, anisakis and latex: Negative. Exercise test with spirometry control: negative without skin lesions. Controlled oral challenge was performed with sulfite at rest with negative results.

Is therefore requested an exercise provocation test and spirometry control after previous oral sulfites, ten minutes after this, she presented pruritus and wheals in neck, scalp, trunk, arms and retroauricular region, requiring antihistamines and oral corticosteroids.

The patient was diagnosed with exercise urticaria dependent sulfites.

Conclusion: We present a case of exercise urticaria dependent sulfites, not having found in the literature so far published similar case. The combined ingestion of sensitising food and exercise is necessary to precipitate symptoms, it can be necessary to perform an exercise challenge test with and without previous ingestion. The treatment is preventive and it is based on avoiding the food or the food allergen some hours before the exercise. When it does not depend on foods it is used a prophylactic pharmacotherapy with antihistamines, cromones or sodium bicarbonate. The patient should be well educated on the use of epinephrine in the event of new reactions.

successfully treated some patients with immunotherapy with allergy vaccine.

Method: We prospectively evaluated 25 patients with urticaria, by detailed history, physical exam, complete blood count, thyroid profile and total and specific IgE by skin prick test and or UNICAP *in vitro* IgE for pollens and foods. Those with positive reactions to pollens were started on immunotherapy and were followed regularly and evaluated for symptoms, drugs taken and by visual analogue both by patients and physician.

Results: Twenty-five patients 18 females and 7 males, their ages ranged from 8 years to 56 years average 30.68 years. Duration of urticaria was 10–17 years in six patients, <1 year in 12 patients, and 1–2 years in three patients and less than 6 weeks in four patients, and in four patients the duration was unknown. Urticaria alone diagnosis was in seven patients, urticaria and bronchial asthma and allergic rhinitis was diagnosed in eight patients, and seven patients had urticaria and allergic rhinitis, and two patients had urticaria and asthma and one patient had urticaria, allergic rhinitis and thyroiditis. Four patients lost to follow up and in four patients there was no improvement on immunotherapy. Those who improved, seven patients had visual analogue of 100%, and became asymptomatic, and six patients visual analogue was 80–90% with occasional mild symptoms, and three patients the visual analogue was 70% and one patient the visual analogue was 50%. The success rate of immunotherapy was 81% and those who had 100% improvement was 33%. Those who received allergy vaccine for a longer time had the highest improvement compared to those who received it for a shorter period. Usually some of our patients who show good clinical response ignore follow up appointments. Therefore we suspect that some of our four patients who were lost to follow up may had good clinical response and did not see the need to come back.

Conclusion: Immunotherapy is highly successful in patients with urticaria when there are no other causes except allergy to pollens.

leading often to the prescription of corticosteroids. Discontinuation of the steroid usually results in flare up of the urticaria symptoms, which need to be managed with alternative means to ensure acceptable quality of life. European guidelines indicate modern second generation H1-blockers in higher than conventional doses as drugs of choice for difficult-to-treat patients. British guidelines, however, recommend sedating antihistamine at night in addition to high doses non-sedating antihistamine.

Method: We carried out a double-blind randomised cross-over study to examine these two different approaches: 5 day treatment with 20 mg levocetirizine (Mono) and 5 day treatment with 15 mg levocetirizine + 50 mg hydroxyzine at bed-time (Combo). Patients on 10–30 mg prednisolone equivalent were invited for the study; their steroid was discontinued and the tolerability to doses 20 mg levocetirizine and 200 mg hydroxyzine was assessed on separate days in hospital environment. Primary variable was quality of life (QoL) measured by urticaria specific questionnaire (CU-Q2oL). Secondary variables were physician-assessed symptom scores (0–3) for wheals and itch, night time sleep disturbance and daytime somnolence on separate 100 mm visual analogue scales (VAS). Higher scores indicated worse symptoms. Adverse events (AE), blood hematology and biochemistry were monitored.

Results: Twenty-four patients completed the study (mean age 45 years; range 19–68; 18 women). CU-Q2oL scores decreased significantly, $P < 0.001$, for both Mono and Combo, without significant difference between the 2 regimens, $P = 0.25$. The same pattern occurred for the symptom scores. Nighttime sleep disturbance significantly decreased with both Mono and Combo. Difference emerged in the daytime somnolence, which was significantly more pronounced in the Combo group, $P = 0.026$. No pathological lab and ECG findings were registered. None of the patients experienced serious or severe AE during the trial.

Conclusion: Worsened QoL of patients after discontinuation of steroid treatment can be significantly improved with higher than standard doses of second generation antihistamine levocetirizine. Adding to it at bed-time the first generation hydroxyzine does not seem to have a benefit for the patients but is associated with increased daytime somnolence.

649

Chronic urticaria: successful treated with immunotherapy

Harfi, HA^{1,2}; Ilescas, MM¹

¹National Center of Allergy, Asthma and Immunology, Riyadh, Saudi Arabia; ²Immunology, Al Faisal University, Riyadh, Saudi Arabia

Background: Urticaria is a common allergic problem especially among females. The underlying are numerous and sometimes cannot be identified. Treatment at present is mostly symptomatic. In this report we

650

Effect of two different drug regimens containing higher than conventional doses of levocetirizine in weaning urticaria patients from systemic corticosteroid treatment

Staevska, MT¹; Gugutkova, MD¹; Lazarova, TC¹; Krallmarkova, TZ²; Dimitrov, VD¹; Popov, TA¹

¹Clinical Centre of Allergy, Medical University – Sofia, Sofia, Bulgaria

Background: Chronic urticaria may assume a weary course in some patients

651

Peripheral blood gamma delta T cells in patients with chronic idiopathic urticaria

Khandjian, L¹; Alpan, O¹¹O & O Alpan LLC, Section on Immunopathogenesis, Fairfax, United States

Background: Several small sized clinical studies have shown changes in the percentage of peripheral blood gamma/delta T cells in allergic disorders, in the absence of any correlation with serum total IgE levels. We looked at a large cohort of patients with asthma, food allergies, allergic rhinitis, eosinophilic esophagitis and chronic idiopathic urticaria (CIU), their IgE levels and peripheral blood eosinophil counts to assess if a correlation with the percentage of peripheral blood gamma/delta T cell exist.

Method: We performed a chart review of 71 adult and pediatric patients looking at the percentage of CD3 positive gamma/

delta T cells in peripheral blood. Of these patients, 18 had asthma, 17 had CIU, 18 had allergic rhinitis and 18 had eosinophilic esophagitis. We compared the gamma/delta T cell percentage of these patients to that of 302 normal controls.

Results: Patients with CIU had a lower percentage of gamma/delta T cells ($P = 0.029$) compared to controls. The control group had a mean of 4.9% gamma/delta T cells with a standard deviation of 3.22, whereas the group with CIU had a mean of 2.8% with a standard deviation of 1.66%. We found no correlation between gamma/delta T cell numbers and IgE levels or peripheral blood eosinophil counts. The average gamma/delta T cell percentage of patients with allergic dermatitis was 6.5%, in asthma patients 5.4%, EoE patients 5.2%, rhinitis patients 4.1%. A control of 302 patients without allergic disorders had an average of 5.6% gamma/delta T cells.

Conclusion: Against our predictions, changes in gamma/delta T cell percentages were only seen in subjects with CIU and with a decrease. Gamma delta T cells are abundant in the skin, playing an important role in antibacterial defense, tissue repair and inflammation. Even though these cells are abundant source of IL-4 and play important role in allergic inflammation, to our surprise, we did not see a correlation with the more IgE mediated disorders. One of the explanations for our observations in patients with CIU is that the decrease we see in the blood may be secondary to these cells migrating to the skin. Looking at gamma/delta T cells more closely, to determine if certain subsets such as gamma/delta 1 (located in the dermis) and subset Vgamma9/Vdelta2 (located in the peripheral blood) show any variation suggesting the presence of such a migration in subjects with CIU.

Poster Session 15

Airborne allergens II

653

Corylus pollen season onset, peak and diurnal variation in Vinnitsa, Ukraine

Slobodianiuk, LV¹; Rodinkova, VV²; Motruk, II¹; Mazur, OI¹; Kremenska, LV²; DuBuske, L³

¹Pharmaceutical Chemistry, Vinnytsia National Medical University, Vinnytsia, Ukraine; ²Pharmacy Department, Vinnytsia National Medical University, Vinnytsia, Ukraine; ³Immunology Research Institute of New England, Gardner, MA, United States

Background: *Corylus* pollen (hazelnut) is an international important airborne allergen which has increased from the 1999 when the first monitoring was done in Vinnitsa when rare *Corylus* pollen grains were identified. Hazelnut plants have increased as the nuts are used by confectionery manufacturers around Vinnitsa. There is increasing sensitivity to hazelnut pollen grains due to early seasonal impact and cross-reactivity with other Betulaceae family members including *Alnus*, *Carpinus*, *Betula*.

Method: Pollen counts were obtained at Vinnitsa National Pirogov Memorial Medical University (VNMU) in 2010 through 2012 from March 1 until October 31 on daily basis and in 2012 using a bi-hourly mode volumetric method with a Burkard trap on the roof at 25 m above ground. This study was conducted in association with the European Aeroallergen Network (EAN).

Results: *Corylus* pollen seasonal onset was March 10 to 17 with the earliest onset March 10, 2011, and latest March 17, 2010 and March 14, 2012. Peak days were March 17 to 23. The earliest and lowest peak (49 p.g./m³) was March 17, 2012 and the latest one in 2011 was the greatest (78 p.g./m³). 2010 had a peak of 60 p.g./m³ on March 20. The pollen season lasted to the end of April in 2011 and 2012 and until April 13, 2010.

Diurnal data showed the greatest hazelnut pollen in the air from 3 a.m. to 5 a.m. which decreased amounts during the daytime. The most intense pollination with concentrations greater than 20 p.g./m³ was between March 15 to 31 for all years. Hazelnut pollen allergy symptoms likely increased during this time each year, with cross reactive *Alnus* pollen also peaking at the same time in Vinnitsa.

Conclusion: *Corylus* pollen has become an important airborne allergen in Vinnitsa

recently with intense pollination seen from March 15 to 31 since 2010 each year. This pollen may be increasing due to planting of hazelnut trees for the confection industry. Symptoms due to hazelnut pollen may be increased in some patients due to cross reactivity with *Alnus* which pollinates at the same time in Vinnitsa.

654

Distribution and characteristics of culturable airborne bioaerosols in a Swiss milk powder processing facility

Mandal, J¹; Lehner, A²; Fricker-Feer, C³; Ziegler, D⁴; Dörig, G²; Tomczyk, A⁴; Stephan, R²; Brandl, H¹

¹Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Zürich, Switzerland; ²Institute for Food Safety and Hygiene, University of Zürich, Zürich, Switzerland; ³Hochdorf Nutritec AG, Hochdorf, Switzerland; ⁴Mabritec AG, Riehen, Switzerland

Background: Bioaerosols are part of airborne particles ranging from submicron, <0.2 µm (virus, endotoxins, mycotoxins) to multi-micron, 0.2–50 µm level (viable organisms like fungi, bacteria and pollen grains). These organisms when attached to dust particles can travel around the processing facility and may come in contact with food products, containers, and other food contact surfaces during processing. The aim of the study was to provide important data on the concentration level, composition of culturable airborne microorganisms in a Swiss milk powder processing facility.

Method: The airborne community (bacteria and fungi) were sampled and characterised (concentration level, diversity, seasonal changes) at different (six indoor, one outdoor) sites within a dairy production facility. Air samples were collected at two seasonally different sampling dates, one in February (winter) and the other in July (summer) 2012 using laser particle counter and impaction sampler. The isolates were identified using mass-spectrometric (MALDI-TOF) as well as molecular (sequencing of 16S, 23S rRNA, rpoB genes) based methods.

Results: In general the particle concentration, size distribution and total counts of airborne bacteria and fungi were higher in the winter time than in summer but

remained rather stable within each indoor sampling site at both sampling times (February/July) with two exceptions. Peak (fungal) and bacterial concentrations occurred at the logistic site with mean values at 391 + 142 during winter sampling and 179 + 33 CFU/m³ during the summer sampling. However, the total bacterial counts from the outdoor site varied significantly with a > five time higher concentration observed in the winter time compared to the summer time sampling. Numbers of culturable microorganisms were generally very low (<100 cfu per m³ of air) during milk powder production. The majority of the airborne bacteria belonged to the genera *Bacillus* and *Staphylococcus* but a total of 25 different (gram positive as well as gram negative) genera were identified in the air. *Penicillium camemberti*, *Penicillium glabrum* and *Scopulariopsis brevicaulis* were identified as airborne fungi.

Conclusion: The data generated in this study will help to evaluate the effectiveness of the plant's sanitation program and the sources of airborne contamination may be determined allowing the increased safety of dairy products.

655

Clinico-immunological characterisation of *Cassia fistula* L. pollen and its allergenic relationship with *Cassia occidentalis* L. pollen

Basak, T¹; Pandey, N²; Bhattacharya, K¹

¹Department of Botany, Visva-Bharati University, Santiniketan, India; ²Mediland Diagnostic Institute, Kolkata, India

Background: The purpose of the present work is to determine the concentration and seasonal periodicity of *Cassia fistula* (CF) pollen grains in the atmosphere of Santiniketan, West Bengal, India with an object to identify the prevalence of sensitisation to CF pollen among the respiratory allergic patients in the city along with the IgE binding proteins of CF pollen and also to observe the allergenic cross-reactivity of CF with airborne *Cassia occidentalis* pollen. This may be used further in clinical and diagnostic purposes for immunotherapy treatment.

Method: An aerobiological survey was performed with a personal Burkard volumetric sampler over a year in Santiniketan of West Bengal. The allergenic potential of the pollen was studied by skin prick tests (*in vivo*), IgE-enzyme-linked immunosorbent assay and IgE specific immunoblotting (*in vitro*) methods. Soluble pollen protein in PBS (pH 7.3) was fractionated in 0–20%(CF1), 20–50%(CF2) and 50–85%(CF3) using $(\text{NH}_4)_2\text{SO}_4$ precipitation. The total protein resolved in 12% SDS-PAGE and IgE immunoblotting was done using sera from six patients having ELISA $p/n > 3.5$. Cross-reactivity of CF pollen with *Cassia occidentalis* was tested by using dot blot method and inhibition immunoblotting.

Results: *Cassia fistula* pollen grains were found to be prevalent in the air of Santiniketan from March to September. Skin prick tests on 243 respiratory allergic patients [Age range 22–61; M/F = 140/103] showed 21.4% positive skin reaction, out of which 6.99% showed +2/3 level of reaction. When SPT was done using ammonium sulphate fractions (CF1, CF2 & CF3) a marked change in skin sensitivity was observed. Total protein was resolved into 12 bands in 12% SDS-PAGE and 25.5 kDa, 43 kDa, 64 kDa, 66 kDa, 69 kDa and 81 kDa proteins were found to have shown positive IgE reactivity through IgE immunoblotting. CF pollen was found to be positively cross-reactive with *Cassia occidentalis* (CO) and two antigenic components of molecular weight 64 kDa and 43 kDa were found to be inhibited by CO pollen antigenic extract.

Conclusion: Three fractionation i.e. CF1 (0–20%), CF2(20–50%) and CF3(50–85%) used for SPT also showed greater positivity, especially the fraction CF3. Six major protein components of *Cassia fistula* have shown sensitisation to atopic patients and two of them have markedly shown allergenic cross reactivity with *Cassia occidentalis* pollen. This information can be helpful for immunotherapy treatment.

and biomedical applications. Their applicability depends strongly on surface modifications, which can decrease potential health hazards. Using a mouse model of allergic lung inflammation, we have shown that SiO_2 and PEG- SiO_2 NPs exert potent adjuvant activity, leading to strong increased lung inflammatory infiltrate after intratracheal (i.t.) instillation of NPs prior to allergen challenge. Yet, surface modifications with amino ($-\text{NH}_2$) or phosphate ($-\text{P}$) groups substantially decreased this adjuvant activity *in vivo*. To evaluate the underlying mechanisms, we assessed if modifications of SiO_2 NPs influence inflammasome activation and trigger selective gene expression.

Methods: LPS-primed mouse bone marrow-derived dendritic cells (BMDCs) from WT and $\text{NLRP3}^{-/-}$ mice were incubated for 5 h with 0.12–10 mg/ml of naked or modified (PEG-, NH_2 - or P-) SiO_2 NPs and inflammasome activation was determined by IL-1 β secretion by both ELISA and western blot. In parallel, mice were sensitised by four repetitive intraperitoneal injections (1 μg OVA/alum), then i.t. instilled with naked or modified SiO_2 NPs (50 $\mu\text{g}/\text{mouse}$), and subsequently aerosol-challenged for 20 min with OVA. 5 days later, gene expression was evaluated by real-time PCR arrays in pulmonary tissue.

Results: SiO_2 and SiO_2 -PEG dose-dependently induced the secretion of the active form of IL-1 β in BMDCs, whereby the effects of SiO_2 were stronger compared to SiO_2 -PEG. These effects were markedly attenuated in mice lacking NLRP3. Moreover, both NPs enhanced Th2 pro-inflammatory milieu in sensitised mice, increased the expression of pro-inflammatory cytokines and chemokines, as well as markers for alternative activation of macrophages and for eosinophil activation. In contrast, non-adjuvant -P and - NH_2 surface modifications diminished inflammasome activation and proinflammatory gene expression.

Conclusions: These observations lead to a better understanding of the mechanisms behind the reduced harmful effects of surface modified SiO_2 NPs.

and type of bio-aerosol may be contributing factors of both allergic and infectious diseases.

Aims of study: 1) To detect type and level of bio-aerosol of each unit in Phramongkutklao Hospital, and

2) To assess the effectiveness of air filters in eliminating or reducing bio-aerosols in each unit.

Study design: Experimental study.

Method: 1) Collect air samples in selected rooms with an air impactor RSC (Biotest) for 2 min (200 l. evacuated air) at 150 cm above the floor.

2) Record room dimensions, number of occupants, people walking in and out, level of humidity and room temperature.

3) Turn on air filter for 3 h and collect air samples with the air impactor at 1, 2 and 3 h after turning on the air filter.

4) Culture media strips in the air impactor were dislodged, sent to the microbiology laboratory and incubated at room temperature for 3 day. Fungal Identification was performed by slide culture preparation technique.

Results: This study examined 9 units, i.e., the ICU, operating room, three inpatient wards, the medical supply unit, waiting lounge, laboratory unit and conference room. Mean (\pm SD.) amounts of bio-aerosols before and after air filtration 1, 2 and 3 h were 171.11 (\pm 77.06), 57.78 (\pm 34.11), 38.33 (\pm 22.78) and 37.14 cfu./m³ (\pm 17.04), respectively. Mean (\pm SD.) levels of bacteria before and after air filtration were 117.78(\pm 41.32), 50.0 (\pm 24.6), 36.67 (\pm 20.01) and 35.71 cfu/m³ (\pm 15.12), respectively. Staphylococcus coagulase negative, Micrococcus, Bacillus and Corynebacterium were the bacteria identified in this study. Mean (\pm SD.) fungi before and after air filtration were 53.3 (\pm 49.10), 7.78 (\pm 13.0), 1.67 (\pm 3.54) and 1.43 cfu/m³ (\pm 3.78), respectively. Aspergillus, Penicillium, Cladosporium and Curvularia were the fungi identified in this study. Level of bio-aerosol depended on the level of humidity, number of occupants and number of people walking in and out.

Conclusion: The bio-aerosol level in each hospital unit depended on the level of humidity, number of occupants and number of people walking in and out. Air filters were effective in reducing bio-aerosol level in a closed space.

656

Surface modifications of SiO_2 nanoparticles diminish inflammasome activation and expression of selected inflammatory genes

Marzaioli, V¹; Thomas, C²; Wiemann, M³; Landsiedel, R⁴; Wohleeben, W⁴; Weichenmeier, I¹; Behrendt, H¹; Traidl-Hoffmann, C¹; Schmidt-Weber, C¹; Groß, O²; Guterth, J¹; Alessandrini, F¹

¹ZAUM – Center for Allergy & Environment, München, Germany; ²Klinikum rechts der Isar, TUM, München, Germany; ³BE R&D, Münster, Germany; ⁴BASF, Ludwigshafen, Germany

Background: Silica nanoparticles (SiO_2 NPs) are widely used in diverse industrial

657

The pilot study of bio-aerosol level and effectiveness of air filters in Phramongkutklao Hospital

Mahakit, P¹

¹Phramongkutklao Hospital, Otolaryngology, Bangkok, Thailand

Background: Bacteria and fungi are common among bio-aerosols leading to allergic symptoms and URTIs. It depends on the allergic reactivity and host defense. Level

658

***Alternaria alternata*: cloning and sequence analysis of the cDNA sequence of a Asp f 6-like allergen**

Gabriel, MF^{1,2}; Postigo, I¹; Suñen, E¹; Gutiérrez-Rodríguez, A¹; Guisantes, JA¹; Tomaz, CT²; Martínez, J¹
¹Department of Immunology, Microbiology and Parasitology, Faculty of Pharmacy and 'Lascazay' Research Center, University of the Basque Country UPV/EHU, Vitoria, Spain; ²Department of Chemistry and CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal

Background: It is well known that *Alternaria alternata* presents a significant level of allergenic cross-reactivity with several other phylogenetically related and non-related allergenic moulds. Nevertheless, to date, some of these phenomena and the participating molecules remain unclear. In one of our previous studies on *Alternaria alternata* a new 24 kDa immunoglobulin E (IgE)-reacting protein, included in the protein family of manganese-dependent superoxide dismutase (MnSOD), was identified.

The aim of this study was to clone the MnSOD gene from *A. Alternata* and to analyse its homology with the GenBank sequences of fungal IgE-binding MnSOD.

Method: A pair of degenerate primers was designed following the homology shared by the published nucleotide sequences of MnSOD of fungal species. The MnSOD of *Aspergillus fumigatus* (Asp f 6) was inserted as the query sequence (Accession no: U53561). The rapid amplification of cDNA ends (RACE) was employed to generate the gene sequence, using mRNA obtained from 5-day-old *A. alternata* culture as template source. Then, RACE products were purified, cloned in pJET vector, sequenced and compared with the sequences available in the GenBank.

Results: A 665 bp cDNA sequence of MnSOD was cloned from *A. alternata* by means of the RACE technique for the first time. Bioinformatic analysis revealed that the translated coding sequence of the cloned cDNA fragment had a conserved C-terminal domain belongs to the iron/manganese superoxide dismutase superfamily. It presented high identities and a closer relation with the IgE-binding MnSOD of *Curvularia lunata* (79%), a phylogenetically close specie to *Alternaria* and shared a lower homology with the allergen Asp f 6, the MnSOD of *Aspergillus fumigatus* (61%), a phylogenetically non-related allergenic mould.

Conclusion: Results suggest that there is a relationship between homology of MnSOD sequence and fungal phylogeny that may support the significantly higher levels of IgE cross-reactivity between *A. alternata* and phylogenetically related moulds than with non-related ones. The obtained cDNA

will enable us to further production of the recombinant MnSOD from *A. alternata* and its molecular and immunologic characterisation. This may provide a tool for a better understanding of sensitisation, pathogenesis, polysensitisation or cross-reactivity phenomena as well as for improving the molecular diagnosis of mould allergy.

659

A subtilisin like serine protease of *Alternaria alternata*: a potentially cross-reactive allergen

Gabriel, MF^{1,2}; Postigo, I¹; Suñen, E¹; Gutiérrez-Rodríguez, A¹; Guisantes, JA¹; Tomaz, CT²; Martínez, J¹
¹Department of Immunology, Microbiology and Parasitology, Faculty of Pharmacy and 'Lascazay' Research Center, University of the Basque Country UPV/EHU, Vitoria, Spain; ²Department of Chemistry and CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal

Background: The presence of multiple close allergen homologues across the fungal kingdom carrying epitope orthologues that are structurally indistinguishable from IgE binding epitopes may raise a considerable problem in diagnostic and classification of fungal allergy. The fungal pan-allergens included in serine proteases (SP) family, that have been reported as major allergens in several fungi, including *Curvularia*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Rhodotorula* and *Trichophyton*, but not yet associated with *Alternaria*, could provide one of the targets in the study of cross-reactivity phenomena caused by mould products. In this regards, we aimed to provide evidence of the existence of the SP gene and its expression in *Alternaria Alternata* and to analyse its homology with the published sequences of fungal SP.

Method: The SP cDNA of *A. alternata* was isolated through a combination of reverse transcriptase-polymerase chain reaction and rapid amplification of cDNA ends (RACE). The degenerated oligonucleotides primers used in these reaction were designed according to the internal cDNA sequence of SP of *Curvularia lunata* (Accession no: EU622631) and the conserved sequences of fungal SP. The isolated cDNA was purified, cloned in pJET vector, sequenced and compared with the sequences available in the GenBank. The subtilisin enzymatic activity of a 10-day-old *A. alternata* metabolic extract was measured spectrophotometrically using the subtilisin-specific substrate, Z-Ala-Ala-Leu-pNA.

Results: In this study, we isolated and cloned a 1104 pb cDNA from *A. alternata*. Bioinformatic analysis revealed that the theoretical SP primary sequence presents serine protease motifs of subtilase family,

showing a high homology with allergenic SP from various fungal sources, namely *C. lunata* (92%), *Cladosporium cladosporioides* (86%), *Penicillium citrinum* (81%), *A. fumigatus* (77%). Moreover, enzymatic activity of subtilisin was detected in metabolic extract of *A. Alternata*.

Conclusion: The results obtained demonstrate the existence of a subtilisin like SP gene in *A. Alternata* and its *in vivo* expression. Moreover, since some reports postulate that in fungi all proteins with >50% identity to known allergens that have been studied are found to be either cross-reactive or allergenic, homology results suggest that this protein have a high probability to be a potential cross-reactive allergen of *A. Alternata*.

660

Aerobiological data in the provinces of L'Aquila and Teramo, Abruzzo

Pace, L¹; de Martinis, M¹; Sansonetti, G¹; Sirufo, M¹; Casilli, M²; Ginaldi, L¹
¹L'Aquila University, L'Aquila, Italy; ²University of L'Aquila, L'Aquila, Italy

Background: Pollen and spore concentrations are an important cause of allergies and many other poor health conditions, the main symptoms of which are usually associated with respiratory problems. Pollen and spore concentrations were collected in L'Aquila and Atri (Italy) from May 2004 to May 2005.

Method: Pollen and spore concentrations measurements were collected in L'Aquila (Italy) and in Atri (Te) from May 2004 to May 2005. A time series of pollen and spore concentrations has been collected using a Lanzoni VPPS 2000 Hirst volumetric sampler (Hirst, 1952) that was installed 20 m above the ground. The sampler has a pump that ensures a constant flow. The flow rate, corresponding to a human mean breath rate of approximately 10 l/min, ensures intake and delivery of particles on a sampling surface. Melinex tape impregnated with a 2% silicon solution was used as trapping surface. Average daily pollen and spore concentration estimations (spores m⁻³) were carried out, the pollen and spores were identified under a Leica light microscope at 4000 magnification on the basis of morphological characteristics; the counting procedure of the bio particles followed the model proposed by Mandrioli et al. (1998).

Results: We report here our investigation on the aerobiological data in two different monitoring station of L'Aquila and Atri (Te), expressed in p/m³.

L'AQUILA. Between May 2004 and May 2005 the highest pollen concentration

was calculated for *Cupressaceae-Taxaceae* with 3337/m³, followed by *Gramineae* with 1263/m³, then *Oleaceae* (genera *Fraxinus* and *Olea*) with 1048/m³ and then *Fagaceae* (with genera *Fagus*, *Castanea* and *Quercus*) and *Urticaceae*.

ATRI During the same period the highest pollen concentration was calculated for *Oleaceae* with 2840/m³ followed by *Cupressaceae* 2464/m³ and *Gramineae* 2424/m³. **Conclusion:** The observed values of daily aerobiological particles can represent an important information that can be used to efficiently manage allergic patients in Abuzzo region.

661

Monitoring of airborne allergenic pollen grains and its impact in a subtropical suburban city

Ghosal, K¹; Bhattacharya, SG¹; Pandey, N²

¹Division of Plant Biology, Bose Institute, Kolkata, India;

²Allergy & Asthma Department, M. P. Birla Medical Research Centre, Kolkata, India

Background: Many airborne pollen grains are being loaded in air in huge amounts with their allergenic properties causing bio-pollution & human health hazards in periodic interval. The purpose of investigation was to determine the peak concentration of dominating pollen grains and their impact on human health.

Method: Bio-monitoring was done by Burkard Volumetric Sampler in a suburban subtropical area near to Kolkata, India (22°82' North, 88° 20' East). Health problems during seasonal change of 500 local people were documented in a survey. Allergy & asthma related hospitalisation was also recorded. Skin Prick Test (SPT), ELISA (Enzyme Linked Immuno Sorbent Assay), SDS-PAGE, IgE specific Immunoblotting were followed.

Results: Two years' pollen calendar showed 37 types of pollen grains where Poaceae were in highest number followed by *Lantana camara*, *Cyperus* sp., *Amaranthaceae-Chenopodiaceae* etc. People reported frequent sneezing & coughing along with respiratory allergy in seasonal changes which showed a positive correlation in peaks of many species in the pollen frequency graph of the 2 years. Age range of 11–30 years & female members showed more susceptibility to seasonal changes than male. The fuels for cooking may provoke increasing of allergy symptoms. Rate of hospitalisation showed parity with blooming period of many dominant species in 2 years. SPT & ELISA confirmed the allergenicity of *Lantana* in air along with other arising species of Poaceae & many others. IgE specific

Immunoblot from SDS-PAGE revealed the most prominent & common reactive protein band of 38 kDa from *Lantana*.

Conclusion: Pollen grains acted major role as bio-pollutants affecting a mass number of people in the study area. Bio-monitoring showing the significant presence of the exotic *Lantana* sp. which grew abundantly in locality replacing native species. Immunobiochemical tests confirmed its allergenic properties. The way of living, poor knowledge of sanitation, less awareness about health hazards led to promote severe level of allergy. The survey, bio-monitoring & hospitalisation rate together proved that pollen grains of *Lantana* are also a promoting factor of respiratory allergy and *Lantana* is a highly allergenic plant.

662

Relationship between *Platanus* tree density and the amount of *Platanus* airborne pollen in an urban environment

Maya-Manzano, JM¹; Silva-Palacios, I²; Gonzalo-Garjón, MA³; Fernández-Rodríguez, S¹; Tormo-Molina, R¹; Cosmes-Martin, P⁴; Blanco-Pérez, RM⁴; Domínguez-Noche, C⁴; Fernández-Moya, L⁵; Alfonso-Sanz, JV⁶; Vaquero-Pérez, P⁶; Pérez-Marin, ML⁶

¹Biology Department, University of Extremadura, Badajoz, Spain; ²School of Agrarian Engineering, University of Extremadura, Badajoz, Spain; ³Allergology Department, Infanta Cristina University Hospital, Badajoz, Spain; ⁴Allergology Department, Virgen del Puerto Hospital, Plasencia Cáceres, Spain; ⁵Allergology Department, Zafra Hospital, Zafra Badajoz, Spain; ⁶IES Donoso Cortés, Don Benito Badajoz, Spain

Background: Plane-trees (*Platanus* sp.) are widely grown as ornamental in cities. *Platanus* airborne pollen is abundant during a short period in early spring and allergy to *Platanus* pollen is a common problem. The aim of our study was to evaluate the amount of *Platanus* airborne pollen in relation to the number of plane-trees planted in four sampling points.

Method: Airborne pollen was monitored in four cities in South West of Spain: Badajoz, Plasencia, Don Benito and Zafra in 2012 using Hirst spore traps. *Platanus* trees were counted and located in a map. Main pollen season was calculated with the 5–95% range. We did not observe pruning trees near the spore traps.

Results: We identified two species of *Platanus* genus, mainly *P. hispanica* and occasionally *P. orientalis*, both only cultivated as ornamentals. More than of 25 000 total ornamental trees were counted in the four cities and plane-trees were the most abundant in the cities studied. Their density by hectare was 3.3 in Badajoz, 4.1 in Plasencia, 1.9 in Zafra, and 2.2 in Don Benito. Pollen season lasted 26 days in Badajoz and Don Benito, 25 days in Plasencia and 29 days in Zafra, from March 12 to April

4. Sum of daily pollen concentrations was 777 (Badajoz), 705 (Plasencia), 6794 (Don Benito), and 633 (Zafra). Peaks of pollen concentration recorded in grains/m³ were 93 (24/3 Badajoz), 76 (27/3 Plasencia), 744 (22/3 Don Benito), and 69 (24/3 Zafra). The number of plane-trees around spore traps in a radius of 100 m was 3 (Badajoz), 0 (Plasencia), 14 (Don Benito), and 10 (Zafra). The diameter of the plane-tree trunks near spore traps in Don Benito was by twice as Zafra ones.

Conclusion: Although plane trees density was in a comparable range in the four cities studied, we found significant differences in the amount of pollen recorded in a sampling point, explicable in that case by the highest number of plane-trees near of the spore trap and also for being trees more developed.

663

Alternaria and *Cladosporium* spore seasonal and bi-hourly distribution patterns in Vinnitsa, Ukraine

Mazur, OI¹; Rodinkova, VV²; Bilous, OS³; Slobodianiuk, LV⁴; Motruk, II⁵; DuBuske, L⁶

¹Pharmaceutical Chemistry, Vinnitsa National Medical University, Vinnitsa, Ukraine; ²Pharmacy Department, Vinnitsa National Medical University, Vinnitsa, Ukraine; ³Vinnitsa Municipal Center of Primary Health Care No 2, Biologists, Vinnitsa, Ukraine;

⁴Pharmaceutical Chemistry Department, Vinnitsa National Medical University, Vinnitsa, Ukraine; ⁵Pharmaceutical Chemistry, Vinnitsa National Medical University, Vinnitsa, Ukraine; ⁶Immunology Research Institute of New England, Gardner, MA, United States

Background: Effective mold allergy control requires knowing the seasonal and daily patterns of mold distribution in the air. This study assesses the most abundant airborne mold spores *Alternaria* and *Cladosporium* in 2009–2012 on a daily basis and in 2012 on a bi-hourly one as well, in Vinnitsa, Ukraine.

Method: Spore counts were obtained at Vinnitsa National Pirogov Memorial Medical University (VNMU) from 2009 to 2011 on a daily basis and in 2012 using a bi-hourly mode employing volumetric methods using Burkard trap on the roof at 25 m above ground.

Results: The average seasonal profile of *Cladosporium* for 2009 through 2012 showed the most abundant mold counts from mid-June through mid-August. The greatest concentrations were recorded between June 15 and July 15 with a seasonal peak of 7000 spores/m³ noted on July 15. An exception was seen in October, 2012 due to unusually warm weather with 5400 spores/m³ recorded on October 8. The period of greatest *Alternaria* mold spores was from July 1 to September 1 with the highest seasonal *Alternaria* mold

concentrations seen from July 1 to 15, with 630 spores/m³ average observed on July 15. However, warm weather in 2012 led to a peak of 670 spores/m³ on September 27, 2012. *Alternaria* spores had an even bi-hourly average seasonal distribution within the day (10–12.5 spores/m³ every 2 h) with a decrease in concentration seen from 1700 to 2300 h to 8.5 to 9.5 spores/m³. *Cladosporium*, in contrast, was characterised by

the well-defined diurnal maximums at 1300 and 1500 h of 150 spores/m³ and 140 spores/m³ respectively whereas throughout the day *Cladosporium* concentrations varied from 85 to 105 spores/m³.

Conclusion: *Alternaria* and *Cladosporium* molds showed greatest distribution during the summer months with peaks in mid – July. However, the period of active *Alternaria* distribution is briefer and later in

comparison with *Cladosporium*. Diurnal data demonstrated a more constant pattern of *Alternaria* mold spore distribution during the day in contrast with *Cladosporium* having the prominent afternoon peaks. Molds, similar to pollens, require assessment of seasonal distribution to optimise allergy control.

Poster Session 16

Aeroallergens and allergy

664

Assessment of CD8+ lymphocytes T proliferation after specific and non-specific stimulation of peripheral blood cells using flow cytometry and CFSE methods

Michalak, A¹; Lewandowska-Polak, A²; Kowalski, ML¹; Grzegorzczak, JŁ¹

¹Department of Microbiology and Laboratory Medical Immunology, Medical University of Łódź Department of Immunology, Rheumatology and Allergy, Medical University of Łódź, Łódź, Poland; ²Department of Immunology, Medical University of Łódź, Rheumatology and Allergy, Łódź, Poland

Background: Method of assessing cell proliferation using flow cytometry enables detection of cell division using CFSE fluorescence dye, which is permanently bound to the protein found in the cytoplasm. Application of the appropriate monoclonal antibodies conjugated with a different dye allows to assess the phenotype of the proliferating cells. Therefore, flow cytometry provides multiparameter analysis, which allows the assessment of both cell proliferation and cell subpopulation.

Method: The study involved seven patients allergic to birch pollen *Bet.v.1* with a clinical diagnosis of allergic rhinitis and conjunctivitis. The control group consisted of 12 healthy subjects. Mononuclear cells were isolated from peripheral blood and cultured for 7 days at 37 °C, 95% humidity, 5% CO₂ in the presence and absence of *Bet.v.1* (100 ng/ml) and PHA. The proliferation of CD8 + T cells were differentiated on the basis of low CFSE fluorescence (CFSE low CD8 +) and CD8 + molecule expression and expressed as proliferation index which is positive ≥ 2 .

Results: Enhanced proliferation of T lymphocytes (CD8 +) was observed after the specific stimulation of *Bet.v.1* and after PHA stimulation. The average ratio proliferation were: 45.12 ± 23.43 ($P < 0.01$) and 83.00 ± 26.38 ($P < 0.01$), respectively. Similar observations of increased proliferation have been made in the group of healthy individuals. The average ratio of proliferation were 8.98 ± 3.72 ($P < 0.01$) after *Bet.v.1* stimulation and 32.81 ± 17.64 ($P < 0.01$) after PHA and were significantly lower than in patients with allergy.

Conclusion: Flow cytometry using CFSE dye may be considered to assess the proliferation of CD8 + T lymphocytes.

665

Anaphylaxis induced by inhalation of *Artemisia vulgaris* pollen: case report

Regateiro, FS^{1,2}; Tavares, B¹; Loureiro, G¹; Pereira, C¹; Segorbe-Luis, A¹

¹Immunology, Coimbra University Hospital Center, Coimbra, Portugal; ²Faculty of Medicine, Immunology, University of Coimbra, Coimbra, Portugal

Background: *Artemisia vulgaris* (A.v.) is a frequent weed in inland Portugal and other southern European countries. Allergy to A.v. pollen usually presents with respiratory symptoms. Pollen polysensitisation is common and sensitisation to A.v. is above 20% among allergic rhinitis patients in Portugal. *Artemisia vulgaris* allergens were shown to cross-react with several food allergens.

Case presentation: A 44-year-old male patient living in a rural area in 'Cova da Beira' region of Portugal was assisted in Emergency during autumn for sudden appearance of palmo-plantar pruritus about 4 h after gardening. No systemic symptoms occurred, namely nasal obstruction, congestion, wheezing or dyspnea, and he was treated with parenteral antihistaminic and corticosteroids.

One month later, again after gardening, he developed widespread urticaria, facial flushing, coughing and pharyngeal constriction. Self-administered oral cetirizine did not improve the condition and he had to be admitted to Emergency.

Careful anamnesis excluded the ingestion of any relevant food allergens before crises. The patient describes the appearance of intermittent mild rhinitis symptoms one year before, having sought no medical help. Previous to that there were no allergic complaints and no familiar history of atopic disease. Palmar dyshidrotic eczema was diagnosed.

To investigate the allergens involved, several tests were performed one month after the last crisis. Skin prick tests (mm) to aeroallergens (GA²LEN panel) were positive to: *Artemisia vulgaris* 12, *Parietaria judaica* 6, *Olea europaea* 5, *Chaenopodium album* 5, and cat dander (histamine 10).

Skin reactivity was negative to all tested food allergens, including those that cross-react with A.v.. Complete blood count with differential, IgG, IgA and IgM were normal. Total IgE was 237 UI/ml. Serum specific IgE levels were: *A. vulgaris* 5.49 kU/l, nArt v 1 5.24 KU/l and *C. album* 0.72 KU/l. Specific IgE for *O. europaea*, ole e 1, rPru p 3, rPru p 4 and CCDs were negative. Complement factors and triptase levels were normal.

Conclusion: Considering the anamnesis, the sensitisation prevalence and the peak season pollen counts of A.v. in this region, the laboratory findings support the diagnosis of anaphylaxis induced by pollen inhalation. However, the involvement of a systemic urticaria syndrome via the palmar dyshidrotic lesions cannot be excluded. Life threatening anaphylaxis after inhalatory contact with *Artemisia vulgaris* is an exceptional allergy presentation to this widespread weed.

666

Acute asthma due to respiratory allergy to ragweed misdiagnosed as idiopathic anaphylaxis

Leru, PM¹; Bozdog, OB²

¹Allergy, Colentina Clinical Hospital, Bucharest, Romania; ²Dermatology, Elias Emergency Hospital, Bucharest, Romania

Background: *Ambrosia Artemisiifolia* (ragweed) is one of the most prominent allergenic weeds in Europe, with pollen grains that induce hypersensitisation in about 20% of exposed population. The most frequent allergic diseases induced are seasonal rhinitis, rhinoconjunctivitis, rhinosinusitis, bronchial asthma, but also angioedema, anaphylaxis and urticaria. Ragweed extension and impact on human health in Romania is increasing, with few available data and has to be evaluated.

Case: We present a case of a 39 years old woman, living in a rural area from Eastern part of Romania, hospitalised for allergological evaluation of a recent episode of acute respiratory failure, diagnosed as anaphylaxis with unknown cause. She has a history of persistent rhinosinusitis, with progressive seasonal autumn aggravation during the last three years, associated with

symptoms of seasonal asthma, that have been ignored. No food, drugs or other triggers of anaphylaxis could be identified. The patient received high doses of oral corticosteroids and antihistamines, with clinical improvement. The allergological evaluation performed in our department after two weeks showed high plasma levels of total IgE and very high specific IgE to ragweed (class 5). Basal spirometry was normal and bronchial provocation test with methacholine was not performed, due to high risk. Skin prick tests to aeroallergens will be done after discontinuation of antihistaminic therapy. Based on the clinical picture, serological tests and the notification of ragweed in that region, we considered the diagnosis of acute asthma due to severe allergy to ragweed, associated with rhinosinusitis. The antiasthma treatment with inhaled corticosteroids, antileukotrienes (montelukast) and antihistamines was started. Specific sublingual immunotherapy to ragweed has to be initiated the next year, if the allergenic exposure can not be avoided.

Conclusions: We consider that the correct diagnosis of allergic asthma instead of anaphylaxis is very important for the patient regarding the long term management and prognosis. This case is illustrative for the importance of environmental evaluation of respiratory allergies and development of aerobiology in Romania.

667

A case of chronic urticaria to cockroach

Bakiri, AH¹; Mingomataj, E²; Gjata, E³; Hyso, E⁴
¹Allergology Department, Hygeia Hospital Tirana, Tirana, Albania; ²Allergology Department, Mother Theresa School of Medicine, Tirana, Albania; ³District Hospital of Lushnja, Lushnje, Albania; ⁴Regional Hospital of Vlora, Vlora, Albania

Background: Exogenous common provoking causes of chronic urticaria may be drugs, foods, preservatives, infections, physical factors, etc. But inhalant allergens and chronic urticaria occurrence is extremely rare as pollens and household allergens often trigger rhinitis, conjunctivitis or asthma. As related to the insects, there are some cases in the literature prescribing cockroach as inducer of hives.

Case report: A 20 year old student was seen in our hospital for urticaria that had begun two months ago. He did not refer of having other allergic symptoms like rhinitis or asthma. The patient had cutaneous elements every day apart from the days he had was away from home. The development of the hives was not related with the physical factors like exposure to low temperature, foods, etc. The skin prick test for inhalant and food allergens resulted posi-

tive for cockroach (4–14 mm). He referred to have plenty of them in their living home. We prescribed him the proper treatment with antihistamines, oral steroids and advised to avoid cockroach. After insect avoidance there was a significant clinical improvement of symptoms.

Discussion: The etiology of chronic urticaria in most of the cases is quite uncertain and quite difficult to determine. In some studies a consideration number of cases with chronic idiopathic urticaria demonstrated sensitivity to cockroach, house dust mites, pollens and animal dander. So we suggest that in patients suffering from chronic urticaria the cockroach, or other mites hypersensitivity would be considered as they may induce chronic urticaria.

Conclusions: Chronic urticaria may be due to aeroallergens like cockroach. In these cases reducing the cockroach allergen exposure in homes would provide significant role in the patient's improvement. Thus, skin prick test with aeroallergens apart from food allergens is an important diagnostic procedure in such cases, where all other investigations have been fruitless.

668

Does aeroallergen trigger anaphylactic reaction? A case based discussion

Cavkaytar, O¹; Soyer, UO¹; Tuncer, A¹; Sekerel, BE¹
¹Faculty of Medicine, Department of Pediatric Allergy, Hacettepe University, Ankara, Turkey

Introduction: Animal epithelia or saliva comprise substantial amount of aeroallergens causing both acute onset hypersensitivity symptoms and/or chronic inflammation ending with allergic rhinitis or asthma. However, allergy and anaphylaxis due to horse allergen exposure is occasionally mentioned. Here we report a patient with a hypersensitivity reaction after horse exposure and discuss the mode of occurrence of reaction.

Case presentations: Nine-year-old asthmatic girl admitted to emergency room due to sudden onset of dyspnea, cough, swelling of upper eyelids, chemosis and widespread urticaria. The symptoms occurred within minutes after she had started to ride a horse for the first time in a studfarm. She was diagnosed as anaphylaxis and treated with adrenaline, antihistamine, systemic corticosteroid, and inhaled salbutamol. Four years ago, she was interpreted as sensitised to cat feather and grass pollen by skin prick tests. In the current evaluation, she was sensitised not only to grass pollen mix (9 × 6 mm) and cat feather (6 × 3 mm), but also to horse feather (16 × 5 mm). Horse epithelia specific IgE

was 6.64 kU/l (class 3). She was prescribed an adrenaline autoinjector.

Discussion: The leading trigger of anaphylaxis in childhood is food. Aeroallergens primarily acting on respiratory system are seldom incriminated for anaphylaxis. However, they may cause conjunctival or cutaneous reactions by acting topically. It is questionable that the reactions occurred after exposure to such a high amount of horse allergen are due to anaphylaxis or they are the sum of local reactions affecting both cutaneous and respiratory systems simultaneously. Anaphylaxis due to horse allergen is very rare both in childhood and adulthood and has been reported in a six year old girl and in an eight year old boy, in whom both respiratory and cutaneous systems were affected recalling locally induced systemic reactions.

Conclusion: Although occasionally mentioned, horse allergy might pose a potential cause of morbidity even in urban settings ending up with multisystem involved reactions in a previously nonsensitized patient. Since it is an aeroallergen the multiple reactions occurred after exposure might still be locally induced multisystemic reactions rather than anaphylaxis.

669

Bronchial asthma by Chinchilla

Burgos Pimentel, AM¹; García Navarro, DJ¹; de Montoro Francisco, AM¹; Mateo Hernández, B¹; Mateos Galván, JM¹; Fernández López, M¹
¹Hospital Central de la Defensa 'Gómez Ulla', Alergia, Madrid, Spain

Background: Chinchilla (*Chinchilla lanigera*, order Rodentia, family Chinchillidae), native to South America, is used in the fur industry and in some laboratory experiment; therefore It can be a potential source of aeroallergens as a pet in the general population. As pet, life expectancy ranges between 10 and 12 years, although up to 20 years in some cases. The cause of allergies to these animals is proteins, which are released to the atmosphere through the skin flakes, saliva or urine thereof.

Method: A 11 year-old male, present with a four years history of dyspnea, cough, with exacerbation in March and April, nasoculares asymptomatic. For the past two years have a chinchilla as a pet. Those dates suffered an exacerbation of respiratory symptoms, with repeated episodes of bronchospasm, which forced him to go to the emergency room several times. After removal of the animal from home, no further symptoms. His Skin tests were performed with epithelia; an extract was prepared with less than a gram of animal hairs. Total and specific IgE for chinchilla

(Phadia), tryptase, ECP, lipocalin specific IgE (Phadia ISAC).

Results: Allergy study: Prick a positive common inhalant pollens, epithelium dog, cat, hamster were positive. Skin tests chinchilla extract negative. Total IgE 161 kU/l, specific IgE Chinchilla epithelium 1.35 ku/l, cat 6.98 kU/l, dog 0.85 kU/l, and hamster 0.99 ku/l, positive to lipocalins IgE. Tryptase 1.35 µg/l. ECP 13.0 µg/l.

Conclusion: 1 A case of IgE-mediated asthma to epithelium chinchilla, evolving favorably upon removal of the animal from home.

2 Five cases have been found in the literature in which the chinchilla epithelium acts as the agent responsible for allergic asthma.

3 We emphasize the presence of cross-reactivity with other mammalian epithelium.

670

Sensitisation to *Dermatophagoides pteronyssinus*, *Dermatophagoides siboney* and *Blomia tropicalis* in children from three family physician's offices

González León, M¹; Castro Almarales, RL²; Álvarez Castelló, M³; Labrada Rosado, A⁴; Oliva Díaz, Y²; Mateo Morejón, M²

¹Familiar Medicine, 'Pedro Fonseca' Docent Polyclinic, Havana, Cuba; ²Allergen Department, National Center of Bioproducts BIOCEN, Bejucal, Cuba; ³Allergology Service, 'Calixto García' University Hospital, Havana, Cuba

Background: Morbidity of allergic diseases mediated by IgE antibodies has increased in the last 40 years. House dust mites have been identified as a major ethiological agent. In Cuba, the most common mites are *Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides siboney* (Ds) and *Blomia tropicalis* (Bt). The goal of this transversal descriptive study fue to assess the allergic sensitisation to these mites in a child population and its relationship to house dust exposure.

Method: One hundred and three allergic children from the Guatao village, a suburban area of Havana city, were selected and classified in two groups according to the degree of exposure to domestic dust, as roughly assessed by a formulary administered by clinical investigators. All of them underwent the skin puncture test in both forearms with standardised allergen extracts: VALERGEN-BT (*B. tropicalis*), VALERGEN-DS (*D. siboney*) and VALERGEN-DP (*D. pteronyssinus*) at 20 000 BU/ml (BIOCEN, Cuba).

Results: Sensitisation to three mites was significantly higher ($P < 0.01$) in the group with greater exposure. In addition, the sensitisation frequency was significantly higher

($P < 0.01$) for *Dermatophagoides* species, specially for Dp (87.9%), as compared to Bt (33.3%). The Dp extract provoked a mean size of the wheal higher than the other products among the positive patients of both exposure groups ($P < 0.04$).

Conclusion: The greater exposure of patients to domestic dust is related to a higher sensitisation intensity and frequency to house dust mites, including *Blomia tropicalis*.

671

Aeroallergens sensitisation profile in a Spanish city population

García Nunez, I¹; Algaba Marmol, MA²; Barasona Villar-ejo, MJ³; Escalona Peña, M⁴; Reina Ariza, E¹

¹Allergy Department, Hospital Quiron, Malaga, Spain; ²District Sanitario Osuna, Centro de Salud 'El Almorón', Ecija, Spain; ³Allergy Department, Hospital Universitario Reina Sofia, Cordoba, Spain; ⁴Hospital Quiron, Malaga, Spain

Background: The most important aeroallergens causing clinical symptoms around the world are house dust mites (HDM), molds, pollens and animals epithelia. Every city has got a different sensitisation profile, being very different between cities separated by a small distance in kilometers. Our aim is to show the aeroallergen sensitisation profile in our patients, the symptoms referred and the percentage of monosensitised/polisensitised patients.

Methods: All patients living in Malaga during the last 10 years coming to our Allergy Department from September to December 2012 referring respiratory symptoms were selected. A clinical report focused in these clinical symptoms was performed, plus skin prick test with the most frequent aeroallergens (HDM, molds, pollens and animal epithelia) and panallergens (palm profilin and peach LTP), total IgE and specific IgE to these aeroallergens.

Results: One hundred and sixty-two patients (74 males and 88 females; medium age 34.88 years) were included, being 92 (56.79%) from 21 to 40 years old. Sixty-six (40.74%) were asthmatic patients. One hundred and six patients (65.43%) were sensitised to HDM, 112 (69.13%) to Olea pollen or 68 (41.97%) to cat dander. Fifteen patients (9.25%) were sensitised to peach LTP and 20 patients (12.34%) to palm profilin. Sixty seven patients (41.35%) presented total IgE higher than 130 KU/l. Twenty patients (12.34%) were monosensitised.

Conclusions: Although Malaga is a typical beach city in Spain, Olea pollen is the most frequent aeroallergen that cause sensitisation.

The patient profile that came to our clinic is a medium aged female with

asthma symptoms in an important percentage of patients.

More studies about relationship between aeroallergen sensitisation and relevance in clinical symptoms are needed to confirm these results.

672

Evaluation of Fel d 1 and Fel d 2 in 65 individuals allergic to cat dander utilising specific IgE testing

Rossi, RE¹

¹Molecular Allergology, Università Vita-Salute San Raffaele, Milano, Italy

Background: Individuals allergic to cat dander are usually to at least 8 allergens when they are exposed to the allergen source (i.e. Fel d 1, 2, 3, 4, 5, 6, 7, 8). Fel d 1, uteroglobin and Fel d 2, serum albumin, can be found in cat hair, dander and saliva.

Method: This retrospective study (during the period 2010–2012) involved 65 patients, mean age 28.3 years, range 4–53 years, with a history of atopic dermatitis; allergic rhino-conjunctivitis, allergic asthma, urticaria. All patients had positive skin test to cat commercial allergen extract Specific IgE antibodies directed to Fel d 1 and Fel d 2 were evaluated by ImmunoCAP-System (Thermo Fisher Scientific).

Results: Sixty-five patients with positive skin test results to commercial cat extract had also serum specific IgE to Fel d 1 (100%), mean 14.97 kUA/l min 0.63 kUA/l, max 100 kUA/l. On the contrary, of 65 patients with positive skin test to commercial cat extract only three patients (4.6%) had specific IgE to Fel d 2 (mean 0.14 kUA/l, min 0.01–0.53 kUA/l).

Conclusion: On the basis of our findings we can conclude that, at least for the majority of allergic population of this geographic area, Fel d 1 is a powerful inducers of IgE production and marker of genuine sensitisation to cat epithelium. On the contrary Fel d 2 is a poor inducers of specific IgE in the majority of cat allergic subjects.

673

Analysis of the reactions of type I hypersensitivity to the nocturnal Lepidoptera in a Costa Rican allergic population, February 2011 – February 2012

Sandi, C¹; Jaikel, D¹; Riggioni, O²

¹Universidad de Costa Rica, San Pedro, Costa Rica;

²Centro de Diagnóstico y Tratamiento de Alergia, Allergy and Immunology, Heredia, Costa Rica

Background: Allergic patients are routinely tested for various allergens including

molds, food, cockroaches and house dust mites; however, there are patients who present allergy symptoms but show negative results in Prick tests, with the available allergens. Therefore, this study aims to determine the percentage of patients sensitised to the nocturnal Lepidoptera and its role as an important environmental allergen.

Method: A retrospective analysis was carried out in which we determined the percentage of patients allergic to nocturnal Lepidoptera, house dust mites (*Blomia tropicalis*, *Dermatophagoides pteronyssinus* and *Tyrophagus putrescentiae*) and insects (*Periplaneta americana* and *Musca domestica*). Also, we analyzed the demographics and symptoms of the patients.

Results: We found that 63.4% of the patients were allergic to house dust mites, 39.2% to nocturnal Lepidoptera, 27.7% to *Periplaneta Americana* and 23.6% to *Musca domestica*. Also, that 2.3% were monosensitized to nocturnal Lepidoptera.

Conclusion: We strongly recommend that Costa Rican allergist constantly test for nocturnal Lepidoptera.

674

Urban vegetation and allergy risk

Thibaudon, M¹; Faucherand, L¹; Oliver, G¹
¹RNSA, Brussieu, France

Background: If chemical pollutants causing air pollution may be subject to control measures, it cannot be the case of biological particles, such as pollen, from vegetation.

In quantitative and qualitative terms, most pollens inhaled by the population and measured by the volumetric downhole pollen traps of RNSA (National Network for Monitoring Aerobiological) are from natural plant species.

On the other hand, in urban area, public garden or park, the composition of the air in terms of pollen is modified by the composition of plant species in their surrounding pollination periods.

Method: At the request of the French Health and Ecology authorities, RNSA published in 2008, an electronic guide 'vegetation in Town' (<http://www.vegetation-en-ville.org>) that aims to inform public and private decision makers on the need to take into account the health component in the choice and maintenance of plant species introduced in urban or peri-urban areas.

This methodological guide contains a number of information about: - Allergy, its clinical manifestations and its impact on everyday life and cost for health.

- Allergies and plants: allergenic potential of pollen species.

- How to act in diversifying species, maintaining in the periods where it is possible to limit the production of pollen.

- For shrubs and trees: indication of species to avoid and proposed substitutions depending on the type of use (hedges, banks, alignment).

- For herbaceous: description of species to avoid depending on their allergenicity: The online guide is downloadable in PDF format, it contains 68 pages of valuable information.

Conclusion: This guide allows local decision makers, landscapers and architects to avoid mistakes which will need many years to recover uniform plantations of birch trees in public parks. It also allows firms to question or ask to RNSA about the allergenic potential of target species and allergic risk potentially induced by revegetation.

675

Acrolein suppresses allergic sensitisation and promotes tumor growth in BALB/c mice

Roth-Walter, F¹; Willensdorfer, A¹; Stremnitzer, C²; Schultz, C²; Diesner, S^{2,3}; Szalai, K⁴; Fazekas, J²; Birnleitner, H¹; Jensen-Jarolim, E^{1,2}

¹Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University of Vienna, University of Vienna, Vienna, Austria; ²Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria; ³Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria; ⁴University of Veterinary Medicine Vienna, Medical University of Vienna, University of Vienna, Messerli Research Institute, Comparative Medicine, Vienna, Austria

Background: Allergic sensitisation has been linked to smoking. Hence, we sought to investigate the contribution of acrolein during nasal sensitisation, since acrolein is one of the components generated in large amounts when smoking cigarettes. Moreover, we investigated impact of acrolein in tumor promotion.

Method: BALB/c mice were nasally sensitised five times in biweekly intervals with KLH alone or in conjunction with moderate amounts of acrolein. Airway hyperreactivity as well as KLH-specific anaphylactic reaction was monitored. Immune response was analyzed on the level of specific antibodies and by cytokine determination from splenocytes after antigen-specific stimulation. Further, D2F2-tumor cells were implanted to the right flank and tumor growth was monitored in mice previously exposed to acrolein or buffer.

Results: In the absence of adjuvant, nasal application of KLH alone was sufficient to induce KLH-specific antibody-titers of

IgG1, IgG2a, IgG2b, IgA and IgE, whereas nasal sensitisation with KLH in the presence of moderate amount of acrolein significantly reduced antibody formation. Similarly, only mice sensitised to KLH, but not in combination with acrolein showed increased airway-hyperreactivity and had a significant drop in body temperature upon allergen challenge. Further only splenocytes of KLH-sensitized mice secreted elevated levels of IL5, IL13, IL10 and IFN- γ . Impaired immune response also led to a significant tumor promotion in mice exposed previously to acrolein.

Conclusion: Nasal application of acrolein prevented nasal sensitisation to KLH and blocked antigen-specific antibody formation. Moreover, tumor growth was significantly promoted in mice previously nasally sensitised to acrolein, but not in controls. Acrolein in smoke decreases the risk of allergic sensitisations and inhibits immune activation, thereby likely affecting susceptibility to infections and promoting tumor growth in smokers.

676

Endogenous biosensors in allergy: cytochrome P450s and the aryl hydrocarbon receptor

Effner, R¹; Pusch, G¹; Brockow, K²; Behrendt, H¹; Schmidt-Weber, C¹; Buters, JTM¹

¹Technical University and Helmholtz Center Munich, ZAUM – Center of Allergy and Environment, Munich, Germany; ²Department of Dermatology and Allergy, Technical University Munich, Munich, Germany

Background: Lipophilic substances like environmental pollutants and drugs can induce their own metabolism by activating xeno-sensitive transcription factors like the aryl hydrocarbon receptor (AhR). This transcription factor induces genes coding for xenobiotic-metabolising enzymes, including cytochrome P450 (P450). P450s are involved in the degradation of lipophilic compounds and the highest abundance of metabolizing enzymes is located in the liver. Little is known regarding the extra-hepatic xenobiotic metabolism. The AhR is receiving much attention in immune cells and therefore metabolising enzymes are investigated here as a system for fine-tuning the immune response.

Method: Human primary mast cells were isolated from foreskins of boys aged 1–15 years ($n = 7$). Basophils, monocytes, B cells, CD8⁺ T cells, CD4⁺ T cells and CD4⁺CD45RO⁺ T cells were isolated from peripheral blood mononuclear cells (PBMCs) of non-atopic individuals ($n = 7$). RNA was analyzed by TaqMan Low Density Arrays (TLDA) specifically designed

for the characterisation of the metabolising system.

Results: Human primary foreskin mast cells, located in the subepidermal dermis show a clear transcription for the AhR-regulated CYP1 family members *CYP1A1* and *CYP1B1*. CD14⁺ cells and basophils revealed a very similar transcription of dif-

ferent genes. *CYP2S1*, *CYP4F3*, *MGST1* and *PTGS2* genes differ most among the lymphoid cell populations. Of all primary cells, B cells were negative for the transcription of *CYP4F22*.

Conclusion: We showed that different immune cells transcribe characteristic patterns of genes coding for metabolising

enzymes. Regulation of different P450s and cytokines in human immune cells by AhR ligands and P450 inhibitors could explain how environmental pollutants predispose the human immune system to become allergic.

Interventions in pediatric allergy: clinical outcomes

679

Soluble CD14, α - and β -defensins in breast milk: association with the emergence of allergy in a high risk population

Savilahti, EM¹; Kukkonen, AK²; Kuitunen, M³; Savilahti, E³; Flora Study

¹Childrens Hospital, University of Helsinki, HUS, Helsinki, Finland; ²Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland; ³Childrens Hospital, University of Helsinki, Helsinki, Finland

Background: Innate immunity factors in breast milk modulate infants' immune responses, and may influence the development of allergy.

Our objective was to study the association of soluble CD14 (sCD14) and defensin levels in breast milk with the emergence of allergy in childhood.

Methods: The randomly selected group of 260 mother-child pairs were part of a randomised, double-blind placebo-controlled trial where 1223 mothers with fetuses in high risk for allergy received for the four last weeks of pregnancy a mixture of four probiotics (Lactobacillus rhamnosus GG, L rhamnosus LC705, Bifidobacterium breve Bb99, and Propionibacterium freudenreichii ssp. shermanii JS) or placebo, and after birth, their child received the treatment for 6 months. Children were followed for the emergence of sensitisation and allergic symptoms for 5 years, with medical examinations at the ages of 2 and 5 years and in the case of allergic symptoms. We measured levels of sCD14, human neutrophil peptide (HNP) 1-3 and β -defensin 2 (HBD2) in colostrum and in breast milk 3 months post-partum with enzyme linked immunosorbent assays.

Results: High soluble CD14 levels in breast milk 3 months post-partum were associated with children developing an IgE-mediated allergic disorder by the age of 5 years ($P < 0.03$). Breast milk sCD14 levels decreased from 0 to 3 months post-partum ($P < 0.0001$). HNP1-3 and HBD2 were detected in colostrum samples. They decreased to levels below detection level by 3 months post-partum. Breast milk HNP1-3, HBD2 or sCD14 levels were not associated with probiotic treatment.

Conclusions: Soluble CD14 in breast milk may influence the emergence of allergy in children with atopic heredity.

680

Molecular spreading in birch allergic children with hay fever – a longitudinal analysis of evolution of the molecular IgE response in childhood

Hatzler, L¹; Panetta, V²; Lau, S¹; Sabina, I³; Bauer, CP⁴; Hoffman, U⁴; Forster, J⁵; Zepp, F⁶; Schuster, A⁷; Keil, T⁸; Wahn, U¹; Matricardi, PM¹

¹Department of Paediatric Pneumology and Immunology, Charite University Medical Centre Berlin, Berlin, Germany; ²L'Altrastatistica srl, Consultancy and Training, Biostatistics Office, Rome, Italy; ³Department of Pulmonary and Allergy, University Children's Hospital Munich, LMU, Munich, Germany; ⁴Department of Pediatrics, Technical University of Munich, Munich, Germany; ⁵Department of Pediatrics St. Hedwig, St. Josefs Hospital, Freiburg, Germany; ⁶Department of Pediatrics and Adolescent Medicine, Johannes Gutenberg University Medical Centre, Mainz, Germany; ⁷Department of Pediatric Cardiology and Pneumology, Heinrich-Heine-University, Düsseldorf, Germany; ⁸Charite University Medical Centre Berlin, Institute of Social Medicine, Epidemiology and Health Economics, Berlin, Germany

Background: The concept of molecular spreading, i.e. the sequential IgE sensitisation against the molecules of an allergenic source, could be shown for children with grass pollen related hay fever. In order to test whether this phenomenon can also be observed in other airborne allergies we have analyzed the data of children from the German Multicenter Allergy Study (MAS) birth cohort with seasonal allergic rhinitis against birch pollen (SARb).

Method: The MAS study, a prospective birth cohort study starting in 1990, included 1314 children in 5 German cities. During the first 13 years of follow-up the children underwent eight blood drawings. Symptoms have been assessed each year according to a standardised ISAAC questionnaire. 820 subjects with complete clinical data of at least eight follow ups have been included in the analysis. Children with symptoms of allergic rhinitis and conjunctivitis in April (and/or March for Southern-West Germany) at least three times in the period between 3 and 13 years of age or at least two times from 11 to 13 years of age have been defined as SARb cases. Blood samples with positive IgE (≥ 0.35 kU_A/l) detected with ImmunoCAP[®] (Thermo Fisher Scientific) have been tested with the ISAC[®] allergen microarray (Thermo Fisher Scientific).

Results: Among 820 children 94 subjects developed SARb. A positive IgE response

could often be detected before the disease onset. In children suffering from SARb a progressive IgE response against birch pollen was observed

(i) on extract level reflected by rising IgE levels against birch extract as well as

(ii) on molecular level showing an increase of the number of sensitisations against birch pollen molecules.

Bet v 1 was detected in the preclinical phase in most of the children. The prevalence of sensitisations to the other molecules Betv2 and Betv4 which are available on the ISAC[®] increased with time but within a slower trend and lower incidence compared to Betv1. A typical sequence with Betv1 as the first recognised molecule, followed by Betv2 and then Betv4, was confirmed in the analysis of the within-patients individual sensitisation progression performed with three different approaches (scoring system, hierarchic incidence, Kaplan-Meier curves).

Conclusion: The sensitisation against birch pollen can start years before onset. With respect to the three available molecules on the ISAC[®] it could be shown that with the progression of the disease the molecular IgE response gets more and more complex (molecular spreading).

681

Gene-environment interaction between CD14/TLR4 polymorphisms and use of antibiotics in infancy increases the risk of allergic rhinitis in Korean pre-school children

Park, KS¹; Lee, HY¹; Seo, J-H²; Kim, H-Y³; Kang, M-J⁴; Kwon, J-W⁵; Kim, B-J⁶; Lee, S-Y⁷; Hong, S-J³

¹Presbyterian Medical Center, Jeonju, Korea; ²Korea Cancer Center Hospital, Seoul, Korea; ³Asan Medical Center, University of Ulsan College of Medicine, Seoul; ⁴University of Ulsan, Asan Institute for Life Sciences, Seoul, Korea; ⁵Seoul National University Bundang Hospital, Seoul, Korea; ⁶Inje University Haeundae Paik Hospital, Busan, Korea; ⁷Hallym Sacred Heart Hospital, Hallym University College of Medicine, Anyang, Korea

Background: Antibiotics use by newborns alters infant microbiota profiles including long-term reduction in microbiota diversity. We investigated the interaction between CD14 -159C/T (rs2569190) and TLR4 +8595C/T (rs1927911) polymorphisms and use of antibiotics in infancy on the development of allergic rhinitis in Korean pre-school children.

Method: A cross-sectional study ($n = 933$) aged 3–5 years was conducted with ISAAC questionnaire. Polymorphisms of CD14 and TLR4 were genotyped by TaqMan assay.

Results: Antibiotic use during infancy was significantly associated with allergic rhinitis (AR) diagnosis (aOR 2.030, 95% CI 1.429–2.884). When we analyzed interaction between use of antibiotics and genotype of the CD14 and TLR4, there was a significant association with development of allergic rhinitis. Children using antibiotics in infancy and TT or TC+CC genotype of the CD14 AR diagnosis had increased (aOR 3.244, 95% CI 1.615–6.516 and aOR 2.474, 95% CI 1.326–4.619, respectively) compared with children having TT genotype of the CD14 and no antibiotics use. Children using antibiotics in infancy and CC or CT+TT genotype of the TLR4 had increased AR diagnosis (aOR 2.872, 95% CI 1.425–5.787 and aOR 2.119, 95% CI 1.129–3.978, respectively) compared with children having CC genotype of TLR4 and no use of antibiotics.

Conclusion: The use of antibiotics in infancy is important risk factors for the development of allergic rhinitis in pre-school children. The interaction of gene (CD14 –159C/T and TLR4 + 8595C/T polymorphism) and use of antibiotics in infancy may influence the development of allergic diseases.

683

Association between asthmatic/rhinitic symptoms and home exposure to mould/dampness in eight Italian cities

Simoni, M¹; Canciani, M²; Carrer, P³; Baldacci, S¹; Biggeri, A⁴; Indinnimeo, L⁵; Brunetti, L⁶; Cibella, F⁷; Pini, C⁸; Viegi, G^{1,7}

¹CNR Institute of Clinical Physiology, Pulmonary Environmental Epidemiology, Pisa, Italy; ²Udine University, Udine, Italy; ³Milan University, Milano, Italy; ⁴Florence University, Florence, Italy; ⁵Rome University, Rome, Italy; ⁶Bari University, Bari, Italy; ⁷CNR Institute of Biomedicine and Molecular Immunology, Palermo, Italy; ⁸ISS, Rome, Italy

Background: In the framework of a study (in progress) on health effects of indoor air quality in the Italian schools, data concerning home characteristics have been also collected.

Methods: We assessed the effects of home exposure to mold/dampness on past year asthmatic/rhinitic symptoms in 2036 schoolchildren (51% males, mean age 10 ± 1.8) from 8 cities (Udine, Sondrio, Milan, Pisa, Rome, Bari, Cagliari, Palermo). Data are from a questionnaire filled in by the parents.

Results: Diagnosis of asthma and rhinitis are reported for 7% and 11% of the children, respectively (3% only asthma, 7%

only rhinitis, 4% both). About 21% of the children are exposed to passive smoking at home. Presence of visible moulds on the walls, mould odor, and dampness/condensation on the windows in winter, are reported in 18%, 6% and 24% of cases. Reported prevalences are 7.9% for wheezing, 22.5% for nocturnal dry cough, 10% for breathlessness, and 20.3% for frequent nasal problems. Regression analyses accounted for gender, age, passive smoking, asthma/rhinitis diagnosis and familiarity, showed the following significant associations: visible mould with breathlessness (odds ratio, OR 2.01, 95% Confidence Interval, CI 1.38–2.92); mould odor with nocturnal dry cough (OR 1.88, 95% CI 1.23–2.86) and breathlessness (OR 2.72, 95% CI 1.59–4.64); dampness/condensation on the windows with nocturnal dry cough (OR 1.27, 1.00–1.64), breathlessness (OR 1.51, 95% CI 1.06–2.15), and frequent nasal problems (OR 1.33, 95% CI 1.01–1.76). Visible moulds and mould odor were also related, at borderline significance, to frequent nasal problems (OR 1.32, 95% CI 0.97–1.80, and OR 1.52, 95% CI 0.93–2.48, respectively).

Conclusion: These preliminary analyses confirm that not only reported visible mould/dampness, but also mould odor, are associated with asthmatic/rhinitic symptoms.

684

Is nasal provocation test important for children with non-allergic rhinitis

Duman, H¹; Bostanci, I²; Ozmen, S²

¹Pediatric Allergy, Dr. Sami Ulus Research and Training Hospital of Women's and Children's Health and Diseases, Ankara, Turkey; ²Dr. Sami Ulus Research and Training Hospital of Women's and Children's Health and Diseases, Ankara, Turkey

Background: Local allergic rhinitis is characterised by the local production of specific IgE antibodies in the nasal mucosa of patients with symptoms of allergic rhinitis in the absence of a positive skin test and specific IgE. It is aimed in this study to investigate the presence of local allergic rhinitis in children by performing a nasal provocation test with grass mix, DP and DF allergens.

Method: Twenty-eight patients and 30 healthy children were included in the study in a pollen free season. The total symptom score, visual analog scale, nasal eosinophilia and pulmonary function tests were evaluated before and after each NPT.

Results: The nasal provocation test was positive in seven (25%) of the patient group (two with grass mix, and three with DP and two with DF). There was no positivity in any patient in the control group.

In the NPT positive group there was a statistically significant decrease for the nasal flow at the concentration of 10 and 100 IR/ml for DF (respectively $P = 0.026$, $P = 0.031$). In the NPT positive group total nasal resistance for DP was increased at the concentration of 0.1 IR/ml and 10 IR/ml; and for DF at 10 IR/ml and 100 IR/ml (respectively $P = 0.049$, $P = 0.041$, $P = 0.022$, $P = 0.035$).

Conclusion: In our study 25% of those patients with nonallergic rhinitis were diagnosed as LAR. We showed that LAR can also be diagnosed in childhood. Nasal provocation test with anterior rhinomanometry is a noninvasive, cheaper, more repetitive and more helpful means of diagnosis in children. We emphasize that the diagnosis of LAR should be taken into consideration by the pediatricians and children's allergy specialists.

685

Subanalysis of clinical efficacy in children with 6–11 years weighing ≥ 25 kg with persistent allergic rhinitis: rupatadine results

Potter, P¹; Maspero, J²; Vermeulen, J³; Barkai, L⁴; Bailieu, RA⁵; Nemeth, I⁶; Garde, JM⁷; Giralt, J⁸; Domenech, A⁸; Izquierdo, I⁸; Nieto, A⁹

¹University of Cape Town, Cape Town, South Africa; ²Fundacion CIDEA, Buenos Aires, Argentina; ³Parow Research, Cape Town, South Africa; ⁴Gyermekesegszegugyl Kozpont, Miskolc, Hungary; ⁵Centro de Alergia, Mar de Plata, Argentina; ⁶Szent Erzsébet Korház, Jászberény, Hungary; ⁷Universidad de Elche, Elche, Alicante, Spain; ⁸Department of Clinical Development, J Uriach y Cia S.A., Palau Solita i Plegamans, Spain; ⁹Hospital Materno Infantil La Fe, Valencia, Spain

Background: Clinical trials with the newer 2nd generation antihistamines in children under the age of 12 years have been performed previously but further studies are needed in order to show efficacy and safety in the most unfavourable clinical conditions such as persistent allergic rhinitis (PER). Rupatadine oral solution was developed for children with allergic rhinitis in view of its rapid onset of action and its lack of relevant side effects. These advantages were confirmed previously in a phase III study in children 6–11 years.

Objective: To assess the efficacy and safety of rupatadine (RUP) oral solution in a subgroup of children between 6 and 11 years weighing ≥ 25 kg with PER

Methods: A subanalysis was performed from a previous placebo-controlled study carried out in patients between 6 and 11 years diagnosed as PER according to ARIA criteria. This analysis included patients with a positive prick test, weight ≥ 25 kg and basal nasal symptoms score

(including rhinorrhea, nasal blockage, sneezing and nasal itching assessment) ≥ 24 obtained in 4 days throughout the 2-week screening period. Patients were allocated to treatment with either RUP oral solution (1 mg/ml) or placebo solution during 6 weeks. The dose was 5 ml of oral solution. The main efficacy endpoint was the change from baseline of the nasal (4TSS) and with ocular symptoms (5TSS) at 4 and 6 weeks of treatment.

Results: The subgroup analysed was a total of 266 randomised to rupatadine ($n = 131$) or placebo ($n = 135$). The primary endpoint 4TSS showed statistically significant differences between placebo and RUP at 4 ($P < 0.01$) and 6 weeks ($P < 0.01$). 5TSS decreased also at 4 and 6 weeks ($P < 0.01$). All individual symptoms (nasal and ocular) improved at 4 and 6 weeks, mainly nasal congestion, sneezing and ocular symptoms.

Conclusion: Rupatadine oral solution (1 mg/ml) was significantly more effective than placebo in improving nasal and ocular symptoms at 4 and 6 weeks. This is the first clinical evidence of the efficacy of a second H1-antihistamine compound in children with PER.

686

Subanalysis of quality of life in children with 6–11 years weighing ≥ 25 kg with persistent allergic rhinitis: rupatadine results

Potter, P¹; Maspero, J²; Vermeulen, J³; Barkai, L⁴; Baillieu, RA⁵; Nemeth, I⁶; Giral, J⁷; Domenech, A⁷; Santamaría, E⁷; Izquierdo, I⁷; Nieto, A⁸

¹University of Cape Town, Cape Town, South Africa;

²Fundacion CIDEA, Buenos Aires, Argentina; ³Parow Research, Cape Town, South Africa;

⁴Gyermekgyógyászati Központ, Miskolc, Hungary;

⁵Centro de Alergia, Mar de Plata, Argentina; ⁶Szent Erzsébet Korház, Jászberény, Hungary; ⁷Department of Clinical Development, U Jriach y Cia S.A., Palau solita i Plegamans, Spain; ⁸Hospital Materno Infantil La Fe, Valencia, Spain

Background: Clinical trials with the newer 2nd generation antihistamines in children under the age of 12 years have been performed previously but further studies are needed in order to show efficacy and safety in the most unfavourable clinical conditions such as persistent allergic rhinitis (PER). Rupatadine oral solution was developed for children with allergic rhinitis in view of its rapid onset of action and its lack of relevant side effects.

Objective: To assess the quality of life (QoL) of rupatadine (RUP) oral solution in a subgroup of children between 6 and 11 years weighing ≥ 25 kg with PER

Methods: A subanalysis was performed from a previous placebo-controlled study carried out in patients between 6–11 years

diagnosed as PER according to ARIA criteria. This analysis included patients with a positive prick test, weight ≥ 25 kg and basal nasal symptoms score (including rhinorrhea, nasal blockage, sneezing and nasal itching assessment) ≥ 24 obtained in 4 days throughout the 2-week screening period. Patients were allocated to treatment with either RUP oral solution (1 mg/ml) or placebo solution during 6 weeks. The dose was 5 ml of oral solution. The main secondary efficacy endpoint was the assessment of QoL at baseline, 28 and 42 days of treatment by means of Paediatric Rhinoconjunctivitis Quality of Life Questionnaire (PRQLQ).

Results: The subgroup analysed was a total of 266 randomised to RUP ($n = 131$) or placebo ($n = 135$). PRQLQ overall score showed statistical significant differences between RUP and placebo at 4 weeks ($P < 0.01$) and 6 weeks ($P < 0.05$). At 4 weeks, all domains including: nose symptoms, eyes symptoms, practical problems, other symptoms and activity limitation showed statistical significant ($P < 0.05$). At 6 weeks only nasal symptoms ($P < 0.01$) and activity limitations ($P < 0.05$) achieved statistically better values than placebo.

Conclusion: QoL evaluation is a relevant measure of the AR treatment efficacy particularly in children. Rupatadine has been found in this study the first clinical evidence of a H₁-receptor antagonist in children between 6–11 years over 25 kg with PER.

687

Disagreement of different estimates of rhinitis prevalence in pre-school children

Pereira, AM^{1,2,3}; Santos, N^{1,2}; Branco-Ferreira, M^{4,5}; Nunes, C^{4,6}; Fonseca, JA^{1,7,8}; Bousquet, J⁹; Morais-Almeida, M^{2,4}

¹Allergy Department, Centro Hospitalar São João, E.P.E., Porto, Portugal; ²Immunology Department, Hospital CUF-Descobertas, Lisbon, Portugal;

³Department of Health Information and Decision Sciences, Faculty of Medicine of the University of Porto, Porto, Portugal; ⁴Sociedade Portuguesa de Alergologia e Imunologia Clínica, SPAIC, Lisbon, Portugal; ⁵Immunology Department, Centro Hospitalar Lisboa Norte, Lisbon, Portugal; ⁶Centro de Imunoalergologia do Algarve, Centro de Imunoalergologia do Algarve, Portimão, Portugal;

⁷Allergy Unit, Instituto and Hospital CUF Porto, Porto, Portugal; ⁸Faculty of Medicine of the University of Porto, CINTESIS – Centre for Research in Health Technologies and Information Systems, Porto, Portugal; ⁹Department of Allergy and Respiratory Diseases, University Hospital and INSERM, Montpellier, France

Background: There is not a single set of universally accepted and validated definition to assess rhinitis prevalence and different studies adopt diverging questions. The definition used in the ISAAC study is the most frequently used in epidemiological

studies. However, without clinical correlation, misclassification is likely (Kim H, Ann Allergy Asthma Immunol 2012). The aims of this study were 1) to compare the prevalence of current rhinitis in pre-school children using two different definitions (ISAAC's vs. an alternative definition which implies a combination of nasal symptoms), and 2) to evaluate the agreement between these definitions and between them and patient-reported physician-diagnosed rhinitis (PR-PDR) and medication use in the previous 12 months.

Methods: Cross-sectional, nationwide, population-based study of 5018 children aged 3–5 years. Data was collected by face-to-face interview to caregivers using an adaptation of the ISAAC questionnaire. Current rhinitis – ISAAC (CR-I) was defined as the presence of one out of three rhinitis symptoms (repeated sneezing and/or itchy nose, blocked nose for longer than 1 h or runny nose without having a cold or flu), in the last 12 months; current rhinitis – alternative (CR-A) implied the presence of at least 2 out of the same 3 symptoms, in the previous 12 months. The presence of PR-PDR and the use of rhinitis medication were self-reported by the respondents. The percent agreement (%A) was presented and results evaluated with Cohen's kappa (κ).

Results: The prevalence estimate using CR-I was 43.5% [95%CI (42.1–44.9)] and using CR-A 20.4% [95%CI (19.3–21.5)]. The proportion of agreement between these definitions was 77.0% ($\kappa = 0.500$, $P < 0.001$).

Rhinitis medication was used by 11.7% and 11.7% referred PR-PDR; eleven percent ($n = 544$) reported both PR-PDR and medication need. When considering CR-I, the %A with PR-PDR was 67.7% ($\kappa = 0.067$); it was 67.6% with medication need ($\kappa = 0.066$) and 67.1% with PR-PDR combined with medication need ($\kappa = 0.265$). When considering CR-A, the %A were, respectively, 82.5% ($\kappa = 0.051$), 82.9% ($\kappa = 0.052$) and 82.7% ($\kappa = 0.355$).

Conclusion: In the same study sample, the use of different definitions was associated with widely different estimates of rhinitis prevalence. The diagnostic accuracy of these definitions needs to be evaluated against clinical examination.

688

Online medical consultations applied to pediatric allergy

Alvarez-Puebla, MJ; Tabar, AI; García, BE; Echechipia, S; Lizaso, MT; Arroabarren, E
Servicio de Alergología, Complejo Hospitalario de Navarra, Pamplona, Spain

Background: We developed a model of Online Medical Consultations (OMC) between Allergists and Primary Care Pediatricians. We aimed to reduce the numbers of children sent to our Allergy Department whose evaluation would have no added value.

Method: We designed a specific form of OMC linked to the Electronic Medical Record that was filled by pediatrician and emailed to the Allergist. Within 48 h, the allergist answered it with the indication of 'referring' or 'not referring' the patient to our consults. In some instances, the allergist gave advice about patient's treatment and care and, only if these advices were ineffective, the child was referred.

Results: From May/2005 to May/2011, we answered 186 OMC corresponding to children under 14 years. The reasons of OMC were food allergic reactions (FAR) (30%), respiratory symptoms (26%), Drug adverse reactions (DAR) (12%), cutaneous symptoms (11%), Immunotherapy doubts (2%) and miscellany (19%). In 50% of the OMC referral was not needed, specially in those with cutaneous symptoms (71%), Immunotherapy doubts and miscellany (100% each). DAR (63%), FAR (53%) and respiratory symptoms (39%) were the main causes of advice for referral.

Conclusion: OMC implementation is possible in Allergy pediatric consults. OMC improves communication between physicians from Primary and Specialised Care and reduces in 50% referral to specialised care consults. So, OMC can reduce the number of specialized care consults with no added value for patients, decreasing in that way both, direct and indirect health costs.

689

Vitamin D deficiency in Asian children: prevalence and association with total and allergen-specific IgE in the PATCH study

Yao, T-C^{1,2}; Tu, Y-L^{1,2}; Chang, S-W³; Tsai, H-J^{4,5}; Huang, J-L^{1,2}; PATCH Study Group
¹Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan; ²Department of Pediatrics, Chang Gung University College of Medicine, Taoyuan, Taiwan; ³Clinical Informatics and Medical Statistics Research Center, Chang Gung University College of Medicine, Taoyuan, Taiwan; ⁴Division of Biostatistics and Bioinformatics, National Health Research Institutes, Institutes of Population Health Sciences, Maioli, Taiwan; ⁵Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States

Background: New evidence show high prevalence of vitamin D deficiency in many countries and some studies support a possible link between low vitamin D status and atopy-related phenotypes in children. The objective of this study was to determine the prevalence of vitamin D deficiency in a population sample of Asian children and to investigate the association between serum 25-hydroxyvitamin D [25(OH)D] concentrations and total and allergen-specific immunoglobulin E (IgE) levels.

Methods: Serum 25(OH)D concentrations were measured by a new automated electrochemiluminescence-based assay, Elecsys Vitamin D total assay (Roche Diagnostics, Mannheim, Germany), in a population-based sample of 1315 Asian children aged 5–18 years in the Prediction of Allergies in Taiwanese Children (PATCH) study. Serum levels of total and allergen-specific IgE were determined by ImmunoCAP and ImmunoCAP Phadiatop Infant, respectively.

Results: The mean concentration of serum 25(OH)D was 20.4 ng/ml (SD: 7.1 ng/ml). Vitamin D insufficiency (defined as serum 25(OH)D < 30 ng/ml) was observed in 90.3% of children. Vitamin D deficiency (defined as serum 25(OH)D < 20 ng/ml) was present in 51.0% of children. The serum 25(OH)D concentration was not significantly associated with total IgE ($P > 0.05$) or allergic sensitisation, defined as a positive Phadiatop Infant test result, ($P > 0.05$). Older age, female gender, higher body mass index, winter/spring months, and environmental tobacco smoke exposure were associated with low vitamin D concentrations (all $P < 0.01$).

Conclusion: Vitamin D deficiency is remarkably common in this population-based sample of Asian children, suggesting that millions of Asian children living in Taiwan may have suboptimal levels of vitamin D. This population-based study provides evidence against the association of vitamin D status with either total IgE levels or allergic sensitisation in Asian children.

The relationship between vitamin D status and allergic diseases merits further study.

690

Free cortisol in 24 h urine as indicators of the impact of long-term therapy with medium doses of inhaled corticosteroids on the hypothalamic-pituitary-adrenal axis in children with asthma

Baumann, SF¹; Baumann, SS²; Kanacki-Stankovic, S¹; Pakaski, S³; Deanovic, M³
¹Children Department, General Hospital Pancevo, Pancevo, Serbia; ²Medical School of Belgrade, Belgrade, Serbia; ³Health Center Pancevo, Pancevo, Serbia

Introduction: Suppression of hypothalamic-pituitary-adrenal (HPA) axis is a potential systemic effect of inhaled corticosteroids (ICS). The concentration of urinary free cortisol (UFC) is a good indicator of the ICS effects on the HPA.

Objective: The aim of this study is to verify with UFC analysis does long-term use of high doses of ICS (Budesonide, Ciclesonid, Fluticasone) affects the HPA.

Methodology: The study includes 94 children aged 5–17 years (average 10.8) with persistent asthma with the long-term Th, from 3 to 108 months (10.5) with moderate doses of ICS (µg): Budesonide (300–600), Ciclesonide (120–240) and Fluticasone (250–500).

The control group consisted of 73 children without ICS Th, 5–17 years old (11.3). In all children was determined UFC in 24 h urine with CLIA method (Chemiluminescent immunoassay). Reference Values: 26.2–408.3 nM.

Results: Budesonide has a average value of the UFC in nM: 245.7 (range: 9.1–2442.3), Ciclesonide: 302 (24.7–981), Fluticasone: 191.1 (16.3–320, 8), control group 272.4 (30.6–951.4). UFC values below the minimum has 5 (5.32%) children. On the Budesonide 3, Ciclesonide 1, Fluticasone 1. Values above the maximum has 8 (8.51%) children. On the Budesonide 4, Ciclesonide 4, while with Fluticasone isn't registered value above the maximum. The control group was not registered under the minimum value, and 10 (13.70%) children had a value above the maximum. With high values of UFC 11 (61%) children are actively involved in sports, six without and five with the ICS-Th.

Discussion: The lowest and highest values of the UFC is recorded at Budesonide. In children with ICS-Th were recorded sporadically low values of UFC, who are on the border of significance $P = 0.05$, and higher have been recorded in the children who play sports. Serum cortisol in children with low and high values of the UFC was

normal. Clinically, we didn't notice hypercortisolism and suppression of HPA. Budesonide and Fluticasone has a lower average value of the UFC compared to the control group, Ciclesonide higher.

692

Decreased vigorous physical activity in school age children with asthma symptoms: differing gender associations

Sevenoaks, H; Belgrave, D; Lowe, L; Kopec-Harding, K; Simpson, A; Custovic, A; Murray, CS
NHS Foundation Trust, University of Manchester, Manchester Academic Health Science Centre, University Hospital of South Manchester, Manchester, United Kingdom

Background: The rise in prevalence of asthma has coincided with changes in lifestyle and environment. One factor which has been implicated is physical activity levels. Studies investigating physical activity and the association with asthma have reported inconsistent effects. Within a population-based birth cohort study (Manchester Asthma and Allergy Study) we examined the association between physical activity and asthma in children age 11 years.

Methods: Children attending age 11 follow-up underwent body habitus measurements, lung function testing (pre/post bronchodilator spirometry; methacholine challenge) and skin prick testing. Parents completed a validated respiratory questionnaire. Subjects were fitted with an accelerometer [Philips Respironics, Murrsville, USA] to their non-dominant wrist and wore continually for 7 days. Children with idle periods >4 h or failure to complete 7 days were excluded. Hourly activity counts and minutes spent in vigorous activity per hour produced a longitudinal data set. Initial analyses identified significant factors (e.g. gender, fat percentage, day of week) associated with activity. These factors were then incorporated into multi-level linear and Poissonian regression models to assess whether the presence of asthma (answered positively to 2 out of: doctor diagnosis asthma; asthma medication; wheeze in last 12 months), asthma medication use, lung function and atopic state could predict activity levels.

Results: 476 children were fitted with accelerometers. Data was complete in 275 children (male $n = 135$; median age 11.5 years). Boys were significantly more active than girls; female gender predicted 11% less overall activity ($P = 0.03$) and 61% less vigorous activity ($P < 0.01$). Higher body fat percentages predicted less activity ($P < 0.01$), but only in boys. Thus girls and boys were analysed separately. Hourly activity counts were not significantly

associated with asthma or asthma medication use. However, girls with asthma had a 34% reduction in vigorous activity ($P = 0.04$) and those on asthma medications had a 37% reduction ($P = 0.02$) compared to non-asthmatic girls. Only boys who reported using asthma medications with exercise had a significant reduction in vigorous activity (33%, $P = 0.04$). Activity levels were not associated with atopy or lung function.

Conclusion: Diagnosis and treatment of asthma is associated with a significant reduction in the level of vigorous physical activity particularly in girls.

693

The reliability, validity, and responsiveness of TRACK questionnaire in Turkish pre-school children

Buyuktiryaki, B¹; Sahiner, UM¹; Yavuz, ST¹; Cavkaytar, O¹; Arik Yilmaz, E¹; Soyer, OU¹; Tuncer, A¹; Sekerel, BE¹

¹Department of Pediatric Allergy, Hacettepe University Faculty of Medicine, Ankara, Turkey

Background: 'Test for Respiratory and Asthma Control in Kids (TRACK)' questionnaire is the first to measure both the risk and impairment domains of the current guidelines in pre-school children. We aimed to measure the reliability, validity, responsiveness, and minimally important difference (MID) of the Turkish version of the TRACK.

Method: A total of 268 children (69.8% boys) were included in the study. Caregivers filled in three TRACK questionnaires, one at each of three clinical visits (initial, 1st month, and 3rd month). At each visit, physicians determined the control level and the treatment step based on the GINA guideline recommendations.

Results: The internal consistency reliability of the Turkish version of the TRACK questionnaire was found to be 0.74, 0.74, and 0.76 at each of the three visits, respectively (reliability statistics, Cronbach's alpha α). There was a significant difference between the mean TRACK scores of the patients in different asthma control status categories ($P < 0.001$). The test-retest reliability was 0.90. The optimal cut-off scores, according to the Youden index, for uncontrolled and very poorly controlled children, were 80 and 60 points, respectively. The MID mean estimates, determined using the 0.5 SD and 1 SEM distribution-based methodology, were 10.9 and 10.8, respectively.

Conclusion: The Turkish version of the TRACK is an accurate and reliable tool for evaluating asthma control status among pre-school Turkish children. Its

widespread use may help physicians correctly assess control levels among children and may improve quality of life for both patients and their caregivers.

694

Wheezing in young children and development of asthma in school age: use of the Asthma Predictive Index in a subtropical environment

Albuquerque, LA¹; Silva, JM¹; Camara, A¹; Arruda, LK²; Ferriani, VP¹

¹Department of Pediatrics, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil; ²Department of Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

Background: We have evaluated the sensitivity and specificity of the Asthma Predictive Index (API) for development of asthma in 9–11 year-old-children after an index wheezing episode in infancy.

Method: Sixty one of 76 children who had been seen at the Emergency Room for an episode of wheezing in infancy (6–24 m) were included and their parents completed a questionnaire on respiratory symptoms. 52 of them underwent skin prick testing to inhalant and food allergens and 48 of those performed methacholine challenge tests. Children were considered asthmatic if they presented previous physician-diagnosed asthma, or at least one of the following symptoms in the past 12 months: wheezing, cough or chest tightness with exercise, or dry cough without colds, accompanied by bronchial hyperresponsiveness, as detected by a PC20 < 4 mg/ml on methacholine challenge test. A positive API score (at least one major criteria: physician-diagnosed eczema or parental asthma; or 2 of 3 minor criteria: physician-diagnosed allergic rhinitis, wheezing without colds or peripheral eosinophilia $\geq 4\%$) was defined based on information collected when the children were seen for the index episode (between 6 and 24 months). Sensitivity, specificity and confidence intervals (CI) of the API index for the diagnosis of asthma at 9–11y were calculated.

Results: Among the 48 children evaluated at school age, 20 (41.7%) were diagnosed with asthma; 13 of them (65%) had positive API index at the time of the index wheezing episode. Of the 28 wheezing babies that did not develop asthma at 9–11y, only 9 (32.1%) had positive API. Sensitivity and specificity of the API index were 65% (IC = 40.8–84.6) and 67.9% (47.7–84.1), respectively.

Conclusion: Using simple parameters as proposed by the API index, the development of asthma can be reasonably pre-

dicted in wheezing infants living in a sub-tropical environment.

695

Quality of life of children with food protein induced gastrointestinal allergies

Meyer, R¹; Godwin, H¹; Dziubak, R¹; De Koker, C¹;

Gloria, D-O¹; Yerlett, N¹; Skrapac, A-K¹; Shah, N¹

¹Great Ormond Street Hospital, London, United Kingdom

Background: Children with a peanut allergy have been shown to have a worse quality of life (QoL) compared to those with insulin dependent diabetes. No such data is available for children with food protein induced gastrointestinal allergies (FPIGA). We therefore set out to study the impact on QoL of this allergic disorder, compared to healthy children and those with cardiac disorders.

Method: Parents of children (2–12 years) diagnosed with FPIGA at Great Ormond Street Hospital were prospectively recruited to complete the parent proxy PedsQL 4.0 questionnaire. This questionnaire provides a mean score of QoL (0–100) as well as a score on physical, psychosocial, emotional and social functioning and nursery/school for age categories: 2–4, 5–7 and 8–12. We compared our data to a published cohort of healthy children with matched age categories and also compared children ages 2–4 to a published cardiac group.

Results: Thirty four questionnaires were completed. The pooled data indicated that children with FPIGA had a significantly worse QoL than healthy children except for social functioning (Table 1). However, in the 2–4 year olds ($n = 12$) with FPIGA compared to children with cardiac disorders, the QoL was significantly worse ($P < 0.0001$) in all categories, including social functioning.

Conclusion: This study indicates that FPIGA have a significant impact on the QoL, which clinicians need to take into account in their management. The pooled data indicated that social functioning was not impaired when compared to healthy controls, but in the 2–4 age group social functioning was lower than children with cardiac disorders. This is an interesting

Table 1 Survey Results

	Trainees	Final Year Medical Students
Number of respondents	94	184
Mean clinical features score (maximum 20)	11.9	11.2
Mean anaphylaxis features score (maximum 8)	7.0	6.5
Mean management score (maximum 6)	5.1	4.4
Percentage identifying need for admission under paediatrics	85%	86%
Percentage identifying need for allergy clinic follow up	88%	N/A
Percentage who felt competent to teach autoinjector technique	63%	N/A
Percentage who felt confident at recognising anaphylaxis independently	N/A	56%

finding, which indicates that most parents of children with FPIGA perceive their child as having normal social functioning in spite of dietary restrictions which often affect children's socialisation. Although our results may have been influenced by the low number of subjects, it may be that the QoL of children with FPIGA is affected in different ways depending on the age of the child. Further data is being collected to answer these questions.

696

Why is pediatric anaphylaxis not managed according to guidelines?

Harlow, G¹; Richardson, J¹; Dowling, N¹; Makwana, N¹

¹Department of Paediatrics, Sandwell and West Birmingham NHS Trust, West Bromwich, United Kingdom

Background: Anaphylaxis is a severe, life-threatening type I hypersensitivity reaction. NICE issued guidance in December 2011 on the management of suspected anaphylaxis. The authors attributed suboptimal management to inadequate understanding by health care professionals.

We evaluate medical students' and junior doctors' knowledge of anaphylaxis and the management of anaphylaxis in a large UK NHS trust.

Method: 1. A paper questionnaire, based on APLS guidelines, was presented to final year medical students and medical trainees.

Participants were asked to assign twenty clinical features to 'allergy', 'suspected anaphylaxis' or 'neither', and to select suitable management options.

2. Case notes of patients under 17 years old with a final diagnostic code of anaphylaxis were reviewed retrospectively. Their anaphylaxis management was compared to NICE guidance.

Results: The results of the survey are shown in Table 1.

Emergency medicine trainees and medical students scored lower on the recognition of anaphylaxis than General Practice and Paediatric trainees. Medical students, general practice trainees and senior paediatric trainees had the worst scores on the management of anaphylaxis.

Initial anaphylaxis management of the 71 analysed cases was poor; 66% received intramuscular adrenaline (33% pre-hospital, 33% in-hospital), 37% oxygen and 17% intravenous fluids. For the reviewed cases, 66% had a known allergy; 72% were admitted with a reaction to one of their known allergens. Of the children who carry an adrenaline autoinjector, 55% used it correctly during their presenting episode. None of these patients received discharge information meeting full NICE criteria on discharge.

Conclusion: We demonstrate that lack of trainee knowledge translates into suboptimal clinical management of anaphylaxis. Our results support the urgent need for allergy to be reinforced in the national medical curriculum, with the aim of improving anaphylaxis management and doctors' ability to educate patients and relatives.

Table 1 for abstract 695

Category	FPIGA Cohort	SD	Healthy Cohort	SD	P-Value
Total	74.30	15.2	82.70	15.40	0.0015
Physical	75.92	16.6	84.48	19.51	0.0106
Psychosocial	73.40	16.3	81.65	15.22	0.0016
Emotional	67.79	21.3	81.31	16.50	0.0001
Social	82.94	16.3	83.70	19.43	0.8201
School	68.85	19.4	78.83	19.95	0.0031

697

Anaphylaxis planning and preparation in schools following local re-structuring of secondary education

Acomb, H¹; Kerrin, D¹

¹Paediatric Department, Barnsley Hospital NHS Foundation Trust, Barnsley, United Kingdom

Background: There is a GALEN position statement with standards for allergy management in schools. Most schools in the United Kingdom contain students at risk of anaphylaxis, but provision of care varies widely. Our secondary care local allergy service has reported care plan consistency, but not yet explored preparedness of local schools. There has been recent major restructuring of secondary schools in Barnsley (none now under local authority control).

Concerns from school nurses prompted review of allergy care in schools.

Method: Secondary schools approached by email and letter. Structured interview

with nominated school representative. Standardised questions asked regarding standards from position statement. Questionnaire to each schools public health nurse.

Results: Eight of ten schools made contact and were interviewed, all Advanced Learning Centres in new builds, two are mergers of existing schools. Seven schools had written allergy plans available for all allergic students. Three schools had no system in place for staff to readily identify an allergic child. Only three schools had a system using photos. All schools offered staff training in allergy recognition and response, all had emergency medication on site. Only two schools had a policy regarding allergy safety in catering, both were currently providing allergen free meals for specific pupils. One school met all five standards audited, five schools met 4 (range 2–5 standards met.) The two schools that were mergers both did well despite having the most recent opening

dates. Two of the three schools open for the longest achieved the poorest results.

Nurses responses often discussed uptake of staff training only. Two schools reported our audit had stimulated organisational changes prior to interview.

Conclusion: Changes in school organisation, accountability, staff and catering arrangements can all have a significant impact on ensuring safety of allergic children. It was concerning that some schools did not engage with the audit at all, and there was significant variation in preparedness amongst schools. School readiness did not seem adversely affected by merging schools; worse outcomes were seen in schools just moving sites. Moving from local authority control to individualised catering arrangements runs risks of allergy safety being overlooked without clear policies in place. The process of survey and feedback has hopefully raised the profile of this issue at a time when schools may have other priorities.

Poster Session 18

Allergy diagnosis and treatment in children

698

Factors influencing primary care physicians in the treatment of allergic rhinitis

Murgia, N¹; Baldacci, S²; Casciari, C³; Maio, S²; Abbritti, G¹; Viegi, G^{2,4}; Spinozzi, F³; ARGA

¹Section of Occupational Medicine, Respiratory Diseases and Toxicology, University of Perugia, Perugia, Italy; ²Pulmonary Environmental Epidemiology Unit, CNR Institute of Clinical Physiology, Pisa, Italy; ³Laboratory of Experimental Immunology and Allergy, University of Perugia, Perugia, Italy; ⁴CNR Institute of Biomedicine and Molecular Immunology 'A. Monroy', Palermo, Italy

Background: Undertreatment or no treatment of allergic rhinitis is still a problem despite the socio-economical burden of this disease and its relationship with asthma. Some studies are suggesting that primary care physician could play a pivotal role in allergic rhinitis management. The objective of this study is to evaluate factors that could influence primary care physician to undertreat/no treat allergic rhinitis patients.

Method: 518 allergic rhinitis patients recruited by their primary care physicians in Region Umbria, Italy, as a part of the ARGAs study, funded by the Italian Agency of Drug (project no. FARM-JYSSA), completed a questionnaire on allergic rhinitis symptoms and treatment. Undertreatment/no treatment was defined as being inadequately treated or having no treatment despite rhinitis symptoms. Chi-square test and logistic regression were performed to assess risk factors for allergic rhinitis undertreatment/no treatment.

Results: 26.2% of the patients had no treatment despite the symptoms and 13.5% were inadequately treated. Patients who had also asthma (OR 0.47, 95% CI 0.30–0.75) and conjunctivitis (0.44, 95% CI 0.27–0.71) were at lower risk of allergic rhinitis undertreatment/no treatment: in asthmatics this reduction was related mainly to the concomitant asthma treatment (OR 0.19, 95% CI 0.10–0.37). Asthmatics undertreated/no treated for rhinitis had the highest prevalence of rhinitis-related quality of life impairment.

Conclusions: Undertreatment/no treatment for allergic rhinitis is still rather frequent among primary care physicians. The simultaneous presence of asthma and an anti-

asthmatic therapy are able to influence positively physicians to treat adequately their patients. Anyway targeted interventions toward a better knowledge of the importance of allergic rhinitis, even if without asthma, among primary care physicians are needed.

699

Assessing allergy management information needs in primary care based on requests for sIgE determinations

Brakel, TM^{1,2}; de Flokstra-Blok, BMJ^{1,2}; Elberink, JNG^{2,3}; Schuttelea, MLA⁴; Christoffers, WA⁴; Roerdink, EM⁵; van der Molen, T^{1,2}; Dubois, AEJ^{2,5}

¹Department of General Practice, University Medical Center Groningen, Groningen, The Netherlands; ²GRIAC Research Institute, Groningen, The Netherlands; ³Department of Allergology, University Medical Center Groningen, Groningen, The Netherlands; ⁴Department of Dermatology, University Medical Center Groningen, Groningen, The Netherlands; ⁵Department of Pediatric Pulmonology and Pediatric Allergy, University Medical Center Groningen, Groningen, The Netherlands

Background: For many patients suspected of allergy, management is initially and often ultimately the responsibility of general practitioners (GPs). However, previous studies have shown that GPs find that providing such care is difficult, especially when anaphylactic symptoms occur. An area of concern is the interpretation of sIgE tests. The purpose of this study is to obtain insight in characteristics of primary care patients for whom an IgE test was requested, with a view to developing a system for providing additional information to the GP together with the test results.

Method: Patients who were sent to a laboratory for one or more sIgE tests received a questionnaire which was completed at home. This questionnaire was specifically developed and tested to obtain key information about the patients' medical allergy history. Parents reported about their child's allergy.

Results: From the patients ($n = 118$, $M_{age} = 31.7$ years; range 12 months to 81 years) 40% was male. For 55% of the patients no diagnosis had been established. A single allergy diagnosis had been made for 26.5% of the patients and 19% reported to have multiple allergies. Of the patients for whom one or more diagnoses were reported, 15.4% had asthma, 23.9%

rhinitis, 18.8% eczema, 4.3% food allergy, and 6.8% had an unspecified allergy. The main reasons for requesting a sIgE test were rhinitis and asthma, 51% and 26% respectively. Less frequent referrals for IgE testing were: eczema 15%, food related allergy 9%, non-food induced anaphylaxis 1%, and other allergy disorders 18%.

Conclusion: Although previous studies show that GPs feel more comfortable managing asthma and rhinitis than patients with food allergy and anaphylaxis, this is not reflected in the request for sIgE testing. A management support system for GPs should therefore include information on airway allergy as well as anaphylaxis.

700

Frequency of normal serum total IgE in allergic diseases in children

Buterleviciute, N¹; Paltarackiene, V¹; Rudzeviciene, O^{1,2}
¹Vilnius University Faculty of Medicine, Vilnius, Lithuania; ²Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania

Background: The total serum immunoglobulin E (IgE) concentrations are relatively crude method of detecting allergic disorders. Normal values will not exclude the presence of allergic disease. The aim of our study was to evaluate the frequency of normal total IgE in various allergic diseases in children and the frequency of normal total IgE level in children with raised specific IgE levels.

Method: Retrospective observational study was performed. A total of 134 patients (78 boys) 40.8 ± 45.7 months old were enrolled. The majority of patients (45.5%) were with atopic dermatitis only, 14.8% – atopic dermatitis with allergic rhinitis and/or asthma, 10.4% – dermatitis due to ingested food, 9.0% – asthma, 20.3% – other allergic diseases. The total IgE and allergen specific IgE were detected using ImmunoCap (Phadia, Sweden), total IgE was compared with age specific reference ranges.

Results: The total IgE level was normal in 54 (40.3%) patients. Normal serum total IgE level was found in 29 (47.5%) patients with atopic dermatitis only, in 2 (10.5%) children with atopic dermatitis and concomitant allergic diseases, in 11 (78.6%)

children with dermatitis due to ingested food. The total IgE level was normal more frequent in patients with atopic dermatitis ($n = 29$, 47.5%) than in patients with atopic dermatitis and concomitant diseases ($n = 2$, 10.5%) ($P = 0.004$), also in patients with dermatitis due to ingested food ($n = 11$, 78.6%) than in patients with atopic dermatitis ($n = 29$, 47.5%) ($P = 0.04$). In children with increased specific IgE ($n = 57$, 42.5%), normal total IgE was in 8 (14%) children.

Conclusion: The total IgE level was normal significantly more frequent in patients with atopic dermatitis than in patients with atopic dermatitis and concomitant allergic diseases and in patients with dermatitis due to ingested food than in patients with atopic dermatitis. One seventh of children with increased specific IgE levels had normal total IgE.

701

Cesarean section delivery and the development of food sensitisation, food allergy, and atopic dermatitis in infants: COCOA birth cohort study

Lee, S-Y¹; Ahn, K-M²; Kim, KW³; Shin, Y-H⁴; Lee, G-S⁵; Kwon, J-W⁶; Kim, B-J⁷; Kim, H-B⁸; Kim, W-K⁹; Yu, J⁵; Hong, S-J⁵

¹Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Pediatrics, Anyang, Korea; ²Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; ³Yonsei University College of Medicine, Severance Children's Hospital, Seoul, Korea; ⁴CHA University School of Medicine, Seoul, Korea; ⁵Childhood Asthma Atopy Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; ⁶Seoul National University Bundang Hospital, Sungnam, Korea; ⁷Inje University Heundae Paik Hospital, Pusan, Korea; ⁸Inje University Sanggye Paik Hospital, Seoul, Korea; ⁹Inje University Seoul Paik Hospital, Seoul, Korea

Background: It has been hypothesised that changes in the gut microbiota resulting from cesarean section delivery could increase a child's risk of developing allergic diseases. We sought to examine the relation between birth by cesarean delivery and food sensitisation, food allergy and atopic dermatitis in infants.

Method: We examined the relation between mode of delivery and the development of food sensitisation, food allergy, and atopic dermatitis among 412 infants based on a prospective birth cohort study. Food allergy and atopic dermatitis were monitored prospectively by pediatric allergist during the first year of life. At age 1 year, a blood sample was taken for the quantification of specific IgE to milk and egg white.

Results: After adjustment for other covariates, infants born by cesarean section had 2-fold higher odds of atopic dermatitis than those born by vaginal delivery (odds

ratio, 2.15; 95% CI, 1.1–4.2). In multivariate analyses, there were no differences in the prevalence of food allergy and food sensitisation according to mode of delivery.

Conclusion: Cesarean delivery increases the risk for atopic dermatitis in infancy, while delivery mode may not be associated with the development of food allergy. The biologic mechanisms underlying these findings still need to be elucidated.

702

Dynamics of sensitisation to cow's milk and soy proteins in infants with atopic dermatitis during last two decades

Makarova, S¹; Borovick, T¹; Namazova, L²; Gribakin, S³; Sergeeva, S²; Garankina, T²; Gevorkian, A²

¹Scientific Center of Children's Health RAMS, Moscow, Russia; ²Research Center of Children's Health, Russian Academy of Medical Science, Moscow, Russia; ³Russian Medical Academy of Postgraduate Training, Moscow, Russia

Background: Cow's milk proteins (CMP) and egg ovalbumin are the major food allergens in infancy. Sensitisation to gluten, peanuts and soy protein (SP) occupies the second position in the list of the main allergens for infants. We analyzed the dynamics of incidence of CMP and SP allergy in pediatric patients (infants) treated at the Research Center of Children's Health at different periods of time during the last two decades.

Method: Three hundred and Fifty-eight infants with atopic dermatitis (AD) were distributed into five groups according to the studies performed in our Center in different years. 1st group ($n = 92$) was observed in 1989–1992, 2nd group ($n = 86$) in 1990–1994, 3rd group ($n = 68$) in 1995–1998, 4th group ($n = 54$) in 2004–2008 and the 5th group ($n = 58$) was observed and treated in 2008–2011. All groups were comparable by the age and severity of symptoms. The level of specific IgE was detected by ELISA test.

Results: The comparison of results of different years shows that the incidence of detection of specific IgE to CMP was stable in studies 1989–1998 (1st group – 72.7%, 2nd group – 68.6%, 3rd group – 72.9%) and tends to decrease ($P = 0.03$) during recent few years (4th group – 57.4%, 5th group – 53.4%). The lower incidence of IgE-mediated sensitisation to CMP in infants with AD can be explained by active food allergy prevention by means of wide usage of preventive HA-formulas. The incidence of SP sensitisation was 6.8% and 5.8% in 1st and 2nd groups and increased in the middle of 1990s ($P < 0.001$) when soy protein isolate based formulas were widely used but CM protein hydrolysates were not common. The high-

est level of sensitisation to SP was observed in 1995–1998 (3rd group – 26.2%). During the last decade SP sensitisation level decreased to 11.1% and later to 7.4% ($P < 0.001$) which is explained by no use of soy-based formulas during first six months of age of patients.

Conclusion: The incidence of sensitisation to CMP and SP in pediatric patients during last two decades has a typical dynamics. Based on clinical studies performed in our center in different years we come to the conclusion that both incidence of CMP sensitisation and of SP sensitisation has decreased in recent years in comparison to 1990s. A significant decrease of SP sensitisation reflects the efficacy of current nutritional strategies in the management of food allergy in children.

703

The diagnostic challenge of non-allergic skin disease

Muresan, A-M¹

¹Regina Maria Medical Centers, Bucharest, Romania

Background: Allergic skin disease is one of the most common reasons for consultation in an allergy clinic. However, allergy diagnosis is not always confirmed.

Method: We present a few illustrative case reports of non-allergic skin disease, initially considered as 'allergy' either by the patient or by the primary care physician.

Results: The clinical diagnosis were rubella, pityriasis rosea Gilbert, scarlatini-form exanthema, molluscum contagiosum, seborrheic dermatitis and oral lichen planus.

Conclusion: Viral infections of skin and non-atopic/contact dermatitis represent a frequent 'allergy' misdiagnosis. A thorough knowledge of dermatology is often required in allergy praxis and a differential diagnosis should always be considered.

704

Diagnosing hypersensitivity reactions to non-steroidal anti-inflammatory drugs in children

Zambonino, MA¹; Muñoz, C¹; Requena, G¹; Mayorga, C¹; Urda, A¹; Corzo, JL¹; Blanca, M²; Torres, MJ²; FIS-Thematic Networks Co-operative Research Centres: RIRAAF (RD07/0064)

¹Pediatric Service, Carlos Haya Hospital, Málaga, Spain;

²Allergy Service, Carlos Haya Hospital, Málaga, Spain

Background: Hypersensitivity reactions to non-steroidal antiinflammatory drugs (NSAIDs) are the most frequent reported reactions. They can be induced by pharmacological mechanism (cyclooxygenase inhibition), being patients classified as

cross-intolerance (CI), and by specific immunological mechanisms (IgE or T cell), being considered as selective reactors (SR). The aim of the study was to analyse a large group of children with a history of NSAID hypersensitivity that were diagnosed by drug provocation test (DPT).

Method: Sixty three children with a history of NSAIDs hypersensitivity were evaluated by DPT. Patients were classified as CI or SR depending on the acetyl salicylic acid response. The atopic status was assessed by prick tests to a standard of common inhalant allergens and total IgE in serum.

Results: The 68.2% were confirmed as having hypersensitivity, and from this 58.1% classified as CI and 41.9% as SR. From a total of 119 DPT performed, 73 were positive (53.4% positive to Ibuprofen, 37% to AAS, 8.2% to metamizol and 1.4 to paracetamol), being angioedema the clinical symptoms presented in 86.3% of cases alone or accompanied with urticaria. All CI cases tolerated the administration of paracetamol. There was a significant number of atopic subjects in those with CI (72%) compared with those with SR (27.7%) and non-allergic controls (30%).

Conclusion: In children CI hypersensitivity to NSAID is more frequent reaction, being ibuprofen the drug most often involved, the most common entity was angioedema, being atopy frequently associated. DPT resulted to be a safe approach for diagnosing these patients.

706

Diagnosis of nonimmediate allergy to betalactams in children

Caubet, J-C¹; Frossard, C²; Fellay, B³; Eigenmann, P¹

¹Department of Child and Adolescent Medicine, University Hospitals of Geneva, Geneva, Switzerland;

²University Hospitals of Geneva, Geneva, Switzerland;

³Central Laboratories, Cantonal Hospital of Fribourg, Fribourg, Switzerland

Background: Urticarial or maculo-papular skin rashes are frequently observed in children treated by betalactams (BL). Such manifestations are more likely to be related to the underlying infection rather than to an allergic reaction due to the antibiotic (less than 10%). Accurate diagnosis of antibiotic allergy is important not only to prevent life-threatening reactions, but also to avoid unnecessary drug restriction associated with increased resistance and health costs. Based on a large cohort of patients, the aim of this study was to evaluate the utility of clinical diagnostic tests in children developing a benign rash during a BL treatment.

Method: Patients with a history of urticarial or maculo-papular rash during and up to 72 h after a treatment with BL, and a

positive oral provocation test (OPT) with the culprit antibiotic were prospectively recruited at the Geneva University Hospital between 2006 and 2010. Subjects underwent intradermal skin tests (IDT) for BL. In addition, lymphocyte transformation test (LTT) were performed by measuring (3) H-thymidine incorporation after 7 days of incubation with the incriminated antibiotic. Basophils activation tests (BAT) were done according to manufacturer protocol (Buhlmann, Switzerland).

Results: We analyzed data from 14 patients with an antibiotic allergy confirmed by a positive OPT (median age of 4.8 years, range 1.1–12.3) and 82 negative controls (median age of 1.7 years, range 0.5–14.5). Delayed-reading IDT were negative in all tested patients. Immediate-reading IDT were positive in seven patients with a positive OPT (50%) and seven patients with a negative OPT (8.5%). The overall sensitivity determined for immediate-reading IDT was 50% and the specificity was 91.5%. The negative and the positive predictive values were 91.5% and 50%, respectively. Data were available for the LTT in nine patients with a confirmed BL allergy (median age of 2.0 years, range 1.1–8.5) and 15 negative controls (median age of 3.1 years, range 0.5–11.1). We observed an overall higher lymphocyte proliferation rate in allergic patients compared to non-allergic children (mean counts per minute (cpm) of 2352 and 1355, respectively). The BAT were negative in the six tested patients with a positive OPT.

Conclusion: Both skin tests and *in vitro* tests (i.e. LTT and BAT) are of limited value and an OPT should be considered in all children who develop a delayed-onset urticarial or macula-papular rash during treatment with a BL.

707

Stevens Johnson syndrome triggered by combination of clobazam, lamotrigine and valproic acid in a child

Balamtekin, N¹; Kılıç, S²; Arslan, M³; Fidancı, MK⁴;

Yavuz, ST⁵; Yapıcı, AK⁶; Kalman, S⁷

¹Department of Pediatric Gastroenterology and Nutrition, Gülhane Military School of Medicine, Ankara, Turkey; ²Department of Pediatrics, Gülhane Military School of Medicine, Ankara, Turkey; ³Department of Pediatric Neurology, Gülhane Military School of Medicine, Ankara, Turkey; ⁴Department of Pediatric Cardiology, Gülhane Military School of Medicine, Ankara, Turkey; ⁵Department of Pediatric Allergy and Immunology, Gülhane Military School of Medicine, Ankara, Turkey; ⁶Department of Plastic Surgery, Gülhane Military School of Medicine, Ankara, Turkey; ⁷Department of Pediatric Nephrology, Gülhane Military School of Medicine, Ankara, Turkey

Introduction: Stevens-Johnson Syndrome (SJS) is an acute life-threatening dermatosis characterised by conjunctivitis, oral

ulcerations, fever and erythematous macules. The most important etiological factors are infections and drugs including anticonvulsants and non-steroidal antiinflammatories. Clobazam related SJS has been reported rarely in the literature. Herein, we report a case of SJS triggered by combination of clobazam, lamotrigine and valproic acid.

Case report: An 8-year-old boy was admitted to our department with a 10-day history of fever, oral mucosal ulcerations and skin lesions. The patient was under the treatment of valproic acid (350 mg bd) and lamotrigine (50 mg bd) for 4 years due to epilepsy. Because of the increase in seizure frequency, clobazam (10 mg bd) was added to therapy one month ago. Physical examination revealed oral mucosal ulcerations, hemorrhagic crust on the lips, bilateral hyperemic conjunctivas and purpuric lesions on the trunk. Laboratory tests, including CBC, serum electrolytes, liver and renal function tests, urine analysis and sedimentation rate were within normal limits. The patient was diagnosed with SJS and treated with methylprednisolone and antibiotics. All anticonvulsants were withdrawn and the patient was treated with phenytoin for seizures. The lesions progressively resolved in 7 days and methylprednisolone therapy was terminated.

Conclusion: Stevens-Johnson Syndrome has been reported with the use of valproic acid and lamotrigine in the literature. During the treatment of valproic acid and lamotrigine for about four years, no skin rash was reported in our patient, but SJS developed after clobazam was added to the treatment protocol. The risk of SJS may increase in children who undergo combined antiepileptic therapy. Pediatric patients taking multiple antiepileptic drugs should be informed of possible cutaneous adverse effects and treatment should be terminated in case of any cutaneous symptoms including mucous membranes.

708

Prurigo simplex? Papular urticaria?

Akar, HH¹; Tahan, F¹; Balkanlı, S²; Özcan, SS²

¹Department of Pediatric Allergy, Erciyes University School of Medicine, Kayseri, Turkey; ²Department of Pathology, Erciyes University School of Medicine, Kayseri, Turkey

Case report: Subacute prurigo clinically presents as excoriated papules mostly in a symmetrical distribution on the extensor surfaces of the extremities, neck, lower trunk, and buttocks. Some patients may have an atopic background. Exogenous toxic factors such as parasites, bacteria, topically or orally administered drugs

deposited on the skin which can induce itching. Skin manifestations are similar to papular urticaria. A 4-year-old boy presented with a 2-month history of intense pruritus and excoriated papules on trunk and extremities. The percentage of eosinophils was 5.7%. IgE level was normal. Skin Prick Test was negative. Cutaneous manifestations were refractory to treatment. Punch biopsy showed prurigo simplex.

709

The influence of allergen-specific immunotherapy on cognitive functions among hay fever children

Muradova, O¹; Namazova-Baranova, L²; Karkashadze, G²; Maslova, O²; Torshoeva, R²; Tomilova, A²; Alekseeva, A²

¹Department of Cognitive Pediatrics, Federal State Budgetary Institution 'Scientific Centre of Children Health' under the Russian Academy of Medical Sciences, Moscow, Russia; ²Federal State Budgetary Institution 'Scientific Centre of Children Health' under the Russian Academy of Medical Sciences, Moscow, Russia

Background: The prevalence of allergic rhinitis comes up to 15% for children 6–7 years old and up to 30% for teenagers 13–14 years old (MI. Asher et al., 2006) according to the ISAAC program. It is marked out that the children, which suffer for a long time from allergic rhinitis with grave and medium grave condition clinical course, have cognitive functions abnormalities in 95.3%.

Method: The complex estimation of the cognitive function condition for 97 children from 8 to 17 years old suffering from hay fever during remission has been carried out. Depending on ASIT the children has been divided on two groups. The 1st group has contained 54 children without having ASIT previously; the 2nd group has contained 43 children having ASIT previously. Both groups were comparable on age and gender. The general clinical allergist and immunoassay has been carried out for all children under investigation. The improved version of the computer psychophysiological system 'Psychomat' model has been used to estimate the child cognitive function.

Results: The investigation results show that the children from the 1st group suffering from hay fever and not having ASIT have the decreasing of cognitive functions at 61% cases, but the children from the 2nd group having ASIT previously have the decreasing of cognitive functions during remission only at 18.6% cases. The decreasing of cognitive activity in the 2nd group is connected with the long intervals (more than 2 years) between the previously conducted ASIT courses and addition of

grave condition allergic rhinitis. For children from the 1st group with hay fever having the associated all-year allergic rhinitis the abnormality of cognitive functions is detected in 77.7% cases. At this time it is proved that the associated disease as bronchial asthma (53%) and as seasonal bronchial asthma (57%) within the scope of hay fever do not give rise to more evident decreasing of cognitive functions that simply uncomplicated clinical course of hay fever (51%).

Conclusion: The regularity in the cognitive function decreasing depending on previously conducted ASIT to patients with hay fever is discovered. The regularity of influence duration and gravity concurrent disease as allergic rhinitis on the cognitive activity decrease is confirmed. The influence of the hay bronchial asthma and all-year asthma on cognitive functions is refuted without depending on duration and gravity of disease.

710

Comparative analysis of allergic pathology in the structure of somatic diseases in children from radiation-exposed families and in their fathers affected by the Chernobyl accident

Nesterenko, ZV¹; Ivanina, YY²; Dobrokhotova, AV²; Pokryshka, LA³; Pilipenko, IA⁴; Zaitseva, SY²; Lysyh, YV³

¹Department of Pediatrics, State Institution 'Lugansk State Medical University', Lugansk, Ukraine; ²Lugansk Municipal Children's Hospital #2, Lugansk, Ukraine; ³Lugansk Regional Clinical Hospital #2, Lugansk, Ukraine; ⁴Lugansk Regional Children's Hospital, Lugansk, Ukraine

Background: Increased morbidity rates in children affected by the Chernobyl accident make the study of probable follow-up risks of long-term health effects in offspring of persons exposed to radiation (PER) to be of a particular interest.

Aim: To study the frequency of allergic diseases (AD) in the structure of somatic pathology in children from radiation-exposed fathers with conducting comparative analysis with the AD frequency rates and concomitant somatic pathology in the fathers-PER.

Method: The results of retrospective clinical study of pediatric patients aged 0–18, born to radiation-exposed fathers (group A, $n = 74$), their fathers, PER by the Chernobyl accident (group B, $n = 74$) and children of control group (CG, $n = 107$) are presented.

Results: AD were found in all groups: A – 27(36.5%), B – 4(8.1%) and CG – 7 (7.5%). Asthma (A) was more frequently observed in group A – 11(14.9%) patients, allergic skin diseases (ASD) were revealed in 17(23%), 9(12.2%) and 14(13.1%)

patients of groups A, B and CG respectively. Diseases of endocrine system (ES), respiratory (RS), nervous (NS), cardiovascular system (CVS), skin and subcutaneous tissue (SST) occurred more frequently in children of group A – in 27(100%), 25 (92.6%), 24(88.9%), 22(81.5%) and 22 (81.5%) patients respectively, while in their fathers diseases of nervous system (NS), cardiovascular (CVS), urinary (US) and digestive system (DS) took the leading places in the structure of their somatic pathology – 26(96.3%), 24(88.9%), 23 (85.2%) and 22(81.5%) respectively.

Conclusion: 1. AD were 4.8 times more frequent in children of affected parents than in children of CG and 4.5 times more frequent than in their radiation-exposed fathers, that is probably due to role of radiation exposure in the development of AD in the offspring of affected parents in the absence of AD manifestations in their fathers-PER.

2. A and ACD were most frequently diagnosed AD in the offspring of affected parents.

3. Diseases of ES, RS, NS, CVS and SST occurred more frequently in children with AD, while diseases of NS, DS, US in their fathers took leading places in the structure of their somatic pathology.

711

Familial Churg Strauss Syndrome in mother and her daughter

Harmanci, K¹; Konak, N²; Kocak, AK¹
¹Pediatric Allergy and Immunology, Eskisehir Osmangazi University, Eskisehir, Turkey; ²Eskisehir Osmangazi University, Eskisehir, Turkey

Churg-Strauss syndrome (CSS), or allergic granulomatous angiitis, is a rare syndrome that affects small- to medium-sized arteries and veins. However, results of several genetic studies have suggested some predisposing hereditary factors. Until now one familial (two sisters) cases of CSS has been reported.

Case 1 (Mother): A 45-year-old woman acquired moderate bronchial asthma at the age of 32 years that was complicated by sinusitis. In May 2011, at the age of 45 years, she complained about slight fever, weight loss of approximately 12 kg within 6 months, paresthesia and paralysis of the lower legs, dyspnea, and skin eruption. Laboratory tests revealed a leukocytosis of 18,500/mm³, 29.1% of which was eosinophils, and a negative perinuclear anti-neutrophilic cytoplasmic antibody (p-ANCA). Eosinophils in bronchoalveolar lavage fluid was found to be 20%. CSS was diagnosed in accordance with the criteria of the American College of Rheumatol-

ogy. After treatment with corticosteroids and cyclophosphamide, the pulmonary infiltration and skin eruption improved.

Case 2 (Daughter): A 17 year girl acquired moderate bronchial asthma at the age of 12 years. By the age 17 years, her asthma was under control; she complained about weight loss, dyspnea, arthralgia, and sinusitis. Her mother had been diagnosed with CSS before. The daughter came to our clinic to find out whether there is a similar disease like her mother. Laboratory tests revealed a leukocytosis of $19,800/\text{mm}^3$, 69.1% of which was eosinophils, and a negative perinuclear anti-neutrophilic cytoplasmic antibody (p-ANCA) result. An open lung histopathological examination of biopsy specimens taken from alveoli and interstitium infiltration of eosinophils, histiocytes, and erythrocytes was observed, and the pathology has reported the 'eosinophilic vasculitis, Churg-Strauss syndrome in the foreground, collagen vascular diseases can be considered. The patient was given 0.5 mg/kg/day of prednisolone. Significant improvement was seen clinically and radiologically.

Conclusion: Familial cases of systemic vasculitis associated with p-ANCA-positive vasculitis and Wegener granulomatosis have been reported by several authors, CSS pathogenesis is unknown, and there is no evidence that genetic factors influence susceptibility to this disease. We have presented a case of familial CSS in mother and her daughter with atopic-type bronchial asthma and negative perinuclear anti-neutrophil cytoplasmic antibody results.

712

Anaphylaxis in an infant caused by menthol containing cologne

Arikan Ayyıldız, Z¹; Akgül, F²; Yılmaz, S²; Özdemir, D²; Uzuner, N¹

¹Division of Pediatric Allergy, Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey; ²Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey

Case report: Severe allergic reactions to cologne or perfumes are rarely reported. We report the case of an infant who developed anaphylaxis after application of cologne to his face by his mother. An otherwise healthy 2-month-old infant was admitted to the emergency department because of facial edema, shortness of breath and cyanosis after application of menthol containing cologne to his face. On physical examination, edema of face, eyelids and lips and urticarial lesions on cheeks were noted. Cyanosis and respiratory difficulty was apparent. He was treated with intramuscular epinephrine,

methyprednisolone, diphenhydramine and nebulised salbutamol. He was hospitalised for observation and discharged after his urticarial lesions and angioedema regressed. The parents refused any diagnostic evaluation with the cologne or mint/menthol products. Avoidance of menthol containing products is recommended to the parents.

To our knowledge, this is the first case of anaphylaxis caused by a cologne in an infant. There was only one case of anaphylaxis to perfume spray reported in a health care worker. There are allergic reactions of menthol (cyclic alcohol derivative of mint) reported in the literature. Immediate hypersensitivity reactions to menthol ranges from urticaria and rhinitis to asthma. Anaphylaxis to the menthol containing toothpaste has also been reported.

This is a rare case of an infant with anaphylaxis to menthol containing cologne. It emphasizes the possibility of an allergen in a form of cologne and investigation of the contents could make a way to the diagnosis in relevant cases.

713

Acute urticaria at the pediatric age, during year 2012, visit at Department of Dermatology-Allergology, Out-Patient Unit Nr 3, Tirane, Albania

Denizi, V¹; Gjoni, B²

¹Allergology, Out-patient, Unit Nr 3, Tirana, Albania; ²Dermatology, Out-patient, Unit Nr 3, Tirana, Albania

Background: Acute urticaria is caused by IgE Immunology mechanism, characterised by itching and 5% of the pediatric population will develop pruritus during some time in its life. The disease lasts for the short period of time and responds effectively to the elimination of the responsible factor and at the usual antihistaminic therapy.

Method: In our study we examined the children from January 2012 until December 2012 in base of medical history, objective examination, basic and specific laboratory control. We recorded information according to age, sex, seasonal distribution and the cause of the diseases and the appearance of the accompanied angioedema.

Results: We visit 228 children 0–14 age, 98 boys (42.9%) and 130 girls (57.1%). Seasonal acute urticaria appeared 46 children (20.1%) during the autumn, 78 children (34.2%) during the winter, 40 children (17.5%) during the spring, 64 and children (28%) during the summer. The most commonly referred caused of urticaria were food (51.0%), medicines (35.7%) and nygm of insects (13.3%). It was found out that 216 children (94.7%) developed urti-

caria and only 12 children (5.3%) angioedema were hospitalised.

Conclusion: The urticaria diagnosis is usually simple the medical history and the physical examination most of the times are sufficient for the diagnosis. Urticaria is more often in the winter and shows greater frequency in the females than the males. Food allergen is more frequent and direction should be given from specialist's doctors to avoid and minimise the complication.

714

Angioedema of the hands and feet in infants under the age of 6 months: an atypical presentation of cow milk allergy

Mistry, R^{1,2}; Adams, G³; Potter, PC^{2,4}

¹Allergy, Red Cross Children's Hospital, Cape Town, South Africa; ²Allergy Diagnostic & Clinical Research Unit, University of Cape Town Lung Institute, Mowbray, Cape Town, South Africa; ³Red Cross Children's Hospital, University of Cape Town, Allergy, Cape Town, South Africa; ⁴Department of Medicine, Observatory, University of Cape Town, Cape Town, South Africa

Background: Cow's milk allergy (CMA) results from an immunological reaction to one or more milk proteins. The prevalence being between 2.5 and 7% and higher in children than in adults. It usually presents with mild urticaria, gastro intestinal symptoms, rhinitis, eczema or severe and potentially life-threatening anaphylaxis. The reactions which occur in less than 2 h are urticaria, angioedema, and acute flare up of atopic eczema.

Method: We present three infants below the age of 6 months who all presented with isolated angioedema of the hands and feet after first exposure to cow's milk protein. Patient 1 was a 4 month old infant who was exclusively breast fed. On introduction of a formula feed developed angioedema on the face within the first 2 h. This subsequently resolved and after 48 h was brought to the hospital with swollen hands and feet. Patient 2 was a 1 month old who presented with swelling of the hands and feet and no other skin lesions prior to that. On history it was found that 6 days previously he was commenced on a formula feed containing cow milk protein. Patient 3 age 2 months was exclusively breast fed and presented with swelling of the hands and feet within 7 days of the initial exposure of a formula feed containing cow's milk protein.

Results and Conclusion: Immunocap RAST for cow's milk and β lacto globulin was elevated in all three patients. They all responded rapidly to elimination of cow's milk, with no recurrence of the oedema of hands and feet.

Poster Session 19

Studies on epidemiology of allergic diseases

716

Serum 25-hydroxyvitamin D levels and allergic rhinitis/hay fever in Norwegian adults – the HUNT study

Mai, X-M¹; Chen, Y²; Langhammer, A¹

¹Norwegian University of Science and Technology, Trondheim, Norway; ²University of Ottawa, Ottawa, ON, Canada

Background: The impact of low vitamin D status on development of allergic rhinitis/hay fever is unclear. We aimed to study the influence of serum 25-hydroxyvitamin D [25(OH)D] levels on incidence of allergic rhinitis/hay fever.

Method: The study included a random sample from an adult cohort population who participated in the Nord-Trøndelag Health Study (HUNT) surveys 2 and 3 (HUNT2, 1995–97 and HUNT3, 2006–2008) in Norway. Serum 25(OH)D levels were measured in blood samples collected at baseline (HUNT2). In 1351 adults who did not report allergic rhinitis/hay fever at baseline, incident allergic rhinitis/hay fever was identified by having or having had allergic rhinitis or hay fever from questionnaires at follow-up. Allergic rhinitis/hay fever was also defined by a stricter definition based on affirmative answers to questions on allergy at a subsequent interview. Odds ratio (OR) and 95% confidence intervals (CI) were calculated after adjustment for self-history of asthma, family history of allergy, age, smoking, physical activity, socio-economic status, body mass index, and season. The analyses were performed in men and women separately.

Results: 13% (9% and 15% for men and women) of the adults developed allergic rhinitis/hay fever during the follow-up period. Among men, serum 25(OH)D level <50 nM was associated with an increased risk of incident allergic rhinitis/hay fever (OR 2.55; 95% CI 0.99–6.53); each 25 nM reduction of 25(OH)D level was associated with an OR of 1.87 (95% CI 1.19–2.95) for allergic rhinitis/hay fever and an OR of 4.57 (95% CI 1.88–11.11) when the outcome is more strictly defined. We also found that low serum 25(OH)D level was significantly associated with allergic rhinitis/hay fever with a positive allergy condition specially to pollen. However, the associations were not significant in women.

Conclusion: Low serum 25(OH)D levels may play a role in development of allergic rhinitis among men but not women.

717

The effect of relation between the grass pollen and hazelnut sensitisation on the frequency and severity of allergic rhinoconjunctivitis and IgE to *rCor a 8*

Cavkaytar, O¹; Buyuktiyaki, B¹; Kizilpınar, I¹; Arik Yilmaz, E¹; U. Soyer, O¹; Tuncer, A¹; Sackesen, C¹

¹Faculty of Medicine, Department of Pediatric Allergy, Hacettepe University, Ankara, Turkey

Background: The high frequency of pollen sensitisation has been reported among peanut and tree nuts sensitised patients in our previous study. The antigen homology of peanut and hazelnut with betula was previously shown. The aim of this study is to find out the relation of the frequency and the severity of allergic rhinoconjunctivitis (ARC) with grass pollen and hazelnut sensitisation in children.

Method: Seventy children diagnosed with ARC, food allergy or eczema are divided into three groups as ‘only grass pollen sensitised’, ‘only hazelnut sensitised’ or ‘both grass pollen and hazelnut sensitised’. DBPCFC tests determined the status of hazelnut allergy. Symptoms and medications for ARC and asthma were scored from 0 to 3 during the spring season from 1 April to 31 July 2012. Symptom and medication scores for ARC and asthma were analyzed by Area Under Curve, Number Cruncher Statistical System ‘p for trend’, Kruskal-Wallis and Spearman tests. IgE to *rCor a 8* and *rPhl p 4b* were measured by ImmunoCAP method.

Results: ARC symptom scores (ARCSS) and medication scores were lower in ‘both hazelnut and pollen sensitised’ group [162, (98–526)] (median, interquartile range) compared to ‘only pollen sensitised’ group [128, (60–201)] ($P < 0.05$). There was a tendency for decrement of ARCSS among four groups; ‘only pollen sensitised’ [117, (87–388)], ‘both pollen and hazelnut sensitised but tolerant to hazelnut’ [136, (25–182)], ‘both pollen and hazelnut sensitised but allergic to hazelnut’ [74, (35–118)] and ‘only hazelnut sensitised’ [20, (0–52)] ($P < 0.0001$, p for trend). IgE to *rCor a 8*

was higher in ‘both hazelnut and pollen sensitised’ group [9.7, (0.04–7.4 kU/l)] compared to ‘only hazelnut sensitised’ group [0.03 (0.05–0.78)], $P = 0.009$. There was a positive correlation between IgE to *rCor a 8* and both grass pollen mixture IgE ($r = 0.53$, $P = 0.001$) and *rPhl p 4b* IgE ($r = 0.54$, $P = 0.03$). When patients with ARC but without asthma were investigated nasal symptom scores, ARCSS and medication scores were all significantly lower in ‘both pollen and hazelnut sensitised group’ compared to ‘only pollen sensitised group’ ($P = 0.003$).

Conclusion: Grass pollen sensitisation is common among hazelnut sensitised children. Our results denote that the severity of seasonal ARC may change according to the presence of hazelnut sensitisation and its clinical reactivity. Moreover IgE to *rCor a 8* levels have a tendency to be high in children with both hazelnut and grass pollen sensitisation.

718

Systemic inflammation and quality of life in Romanian patient with allergic rhinitis

Bujor, IA¹; Deleanu, D²; Bocsan, IC²

¹Immunology Allergy, University of Medicine and Pharmacy Cluj Napoca, Cluj Napoca, Romania;

²University of Medicine and Pharmacy Cluj Napoca, Cluj Napoca, Romania

Background: Allergic rhinitis is the most frequent allergic disease and it involves local and sometimes systemic inflammation.

The aim of this study was to evaluate systemic inflammation in patient with allergic rhinitis and to see the influence of inflammation markers in quality of life parameters.

Method: Seventy-nine patients were evaluated in our clinic using: total symptoms score, quality of life questionnaire (RQLQ) and visual analog scale (VAS). Serum levels of adhesion molecules (E-selectin, ICAM-1, VCAM-1) were also measured in allergic rhinitis patients.

Result: Medium age in our patients was 30.44 ± 9.9 ; 49.36% were women and 83.54% were from urban area. 55.69% of the pts were sensitised to outdoor and indoor allergens. The majority of the pts (70.88%) had moderate-severe allergic rhinitis.

nititis (MSAR). Number of years since the beginning of the disease was significant higher in pts with MSAR ($P = 0.04$). Serum values of adhesion molecules were significantly higher in allergic rhinitis patients towards healthy subjects for ICAM-1 ($P = 0.001$) and VCAM-1 ($P = 0.001$) but not for E-selectin ($P = 0.764$). Symptoms score had a good and statistical significant correlation with RQLQ ($P = 0.000$) and VAS ($P = 0.000$). Systemic inflammation was not correlated with the severity of the disease and with quality of life scores.

Conclusion: In Romania, allergic rhinitis is a young adult disease from urban area. The most frequent form is moderate-severe allergic rhinitis. The severity of the disease is well correlated with quality of life scores, but not with systemic inflammation.

720

Persistent allergic rhinitis was frequently over-diagnosed by specialists in a nationwide cross-sectional study

Larenas-Linnemann, DE¹; Dinger, H²; Shah-Hosseini, K²; Michels, A²; Moesges, R²; Rodríguez Pérez, N²; Ambríz, M²; Nereida López, D²; Arias, A²; Pizano, L²; Monteverde Maldonado, A²; López, JDR²; Medina, A²; García Imp, DA²; Mexican Study Group on Allergic Rhinitis SPT Sensitivity

¹Allergy, Hospital Medica Sur, Mexico, Mexico; ²IMSIE, University of Cologne, Cologne, Germany

Background: Allergic rhinitis (AR) symptom phenotypes have been described and two classifications exist: the seasonal-perennial (SAR-PAR) one and the ARIA one: intermittent-persistent (INT-PER) and mild-moderate/severe. International treatment recommendations are based on the ARIA classification. How the categorisation of these two compare and relate to a Visual Analogue Severity Scale (VAS) and how their diagnosis differs between patients and allergists has not been described before on the American continent.

Method: Skin prick test positive confirmed AR patients seen nationwide by Mexican allergists, filled in a validated AR questionnaire, demographic data and marked severity on a VAS. Moreover, their AR phenotype was documented by their physician. Pearson's X-squares were used (with Yates's correction) to compare AR phenotype prevalence and Spearman's rho to analyze correlation with VAS.

Results: Of 524 patients, most had INT (56.5%), PAR (82.2%) and moderate-severe (84.7%) AR. However, 57% of the INT-PAR patients was misdiagnosed as persistent-PAR by their physician ($P < 0.0001$). PER is a different phenotype with specific patient's characteristics:

patients have statistically significant longer clinical history, more PAR, more nose and eye symptoms and more severe disease, with a higher VAS-score and only 7% mild symptoms. VAS values ≥ 7.45 relate to PER (sensitivity 68%, specificity 65%). VAS score ≤ 6.2 indicated mild and VAS ≥ 6.4 moderate-severe AR. Just as for adults, for 2–11 year, 12–17 year and 18+year age-groups perennial, intermittent, moderate-severe rhinitis was the most frequent, but children had more INT ($P < 0.01$) and mild ($P < 0.03$) symptoms; adults had more SAR ($P = 0.03$) and less physician's diagnosed asthma ($P < 0.05$). Patients from public health care, where services are without charge, have more INT ($P = 0.016$).

Conclusion: Especially in the PAR group physician's classification of INT vs PER often goes astray, as is seen in other publications in which physicians -not patients- are asked to classify AR. This finding is of significance, as treatment is based on ARIA classification. A simple 5-items patient-directed questionnaire (in multiple languages on ARIA web-site) might help to properly diagnose AR phenotypes. In subjects seen by allergists in Mexico rhinitis symptom phenotypes differ per age-group and between private versus public health care. PER is a more severe phenotype. VAS is useful to evaluate severity.

721

Allergic and non-allergic rhinitis in children: association of symptoms and sensitisation with age, gender, season, and other factors

Back, JH¹; Han, MY¹; Lee, SY²; Kim, WK³; Park, YM⁴; Kim, JH⁵; Ahn, K⁶; Hahm, M-I⁶; Chae, Y⁷; Lee, K-J⁸; Kwon, H-J⁷

¹CHA University School of Medicine, Seongnam, Korea;

²Hallym University College of Medicine, Anyang, Korea;

³Inje University College of Medicine, Seoul, Korea;

⁴Konkuk University School of Medicine, Seoul, Korea;

⁵Sungkyunkwan University School of Medicine, Seoul, Korea;

⁶Soonchunhyang University, Asan, Korea;

⁷Dankook University College of Medicine, Cheonan, Korea;

⁸Korea National Open University, Seoul, Korea

Background: To evaluate the prevalence and clinical characteristics of school children with allergic rhinitis (AR) and non-allergic rhinitis (NAR), and to estimate the effect of gender, age, and aeroallergen sensitisation on the severity of symptoms.

Method: This was a cross-sectional study of 7637 Korean school children who were tested for sensitisation to 19 common aeroallergens. Children with symptoms of rhinitis were classified as having AR or NAR based on skin prick testing.

Results: The overall prevalence of rhinitis was 46.9%, with 59% of these children having AR and 41% having NAR. The

prevalence of children with rhinitis was similar in 6–7 year-olds and 12–13 year-olds ($P = 0.115$). However, the prevalence of AR was greater in older children (23.2% vs. 31.9%, $P < 0.001$), especially seasonal AR (3.2% vs. 5.0%, $P < 0.001$), whereas NAR was more prevalent in younger children (22.9% vs. 15.7%, $P < 0.001$). AR was more common in males (15.8% vs. 11.8%, $P < 0.001$), children exposed to second-hand smoke (15.4% vs. 11.5%, $P = 0.01$), and children with more severe symptoms (2.2 ± 0.7 vs. 1.9 ± 0.6 , $P < 0.001$). Symptoms of AR were more severe in children who were sensitised to more allergens. Relative to children with NAR, those with AR experienced more severe rhinitis, sneezing, rhinorrhea, and itching ($P < 0.001$ for all), but similar severity of nasal obstruction ($P = 0.754$).

Conclusion: The present findings regarding the factors influencing the prevalence and severity of AR and NAR may be helpful that understanding of clinical characteristics and course of disease.

722

Rhinoconjunctivitis and epidemic asthma by guinea pig

García Navarro, DJ¹; Burgos Pimentel, A¹; de Montoro Francisco, A¹; de Membrillo Novales, FJ²; Fonseca Avendaño, J¹; De Mateo Hernandez, B¹; Chivato Perez, T¹; Jiménez Garofano, C¹; Fernández López, M¹; Jareño Esteban, JJ³

¹Allergology, Hospital Central de la Defensa 'Gómez

Ulla', Madrid, Spain; ²Internal Medicine, Hospital

Central de la Defensa 'Gómez Ulla', Madrid, Spain;

³Pulmonology, Hospital Central de la Defensa 'Gómez Ulla', Madrid, Spain

Background: The guinea pig *Cavia porcellus* is a rodent from South America where it is ingested as a typical food. Also used as a laboratory animal, where described up to one third of cases of allergies to these animals among workers, being the guinea pig sensitisation which produces more (up to 31%). The most common symptoms are rhinoconjunctivitis, with or without more severe reactions such as asthma or even cause urticaria, angioedema, contact dermatitis, and anaphylaxis.

Method: We report the involvement of three members of a family of five, caused by the presence of a guinea pig as a pet, causing them those symptoms:

Mother, 42: rhinoconjunctivitis and asthma.

Daughter, 21: rhinoconjunctivitis, asthma, generalised pruritus and contact urticaria.

Son, 19: rhinoconjunctivitis, asthma and contact urticaria.

All them improved after removal of the animal from home, being moved to the next floor, where the mother of the new

family begins with rhinoconjunctivitis symptoms in the presence of the animal.

Skin tests with epithelia total and specific IgE (Thermofisher), ECP and basal tryptase.

Results: Mother, positive skin tests to dog dander 5.5 mm, total IgE 1773 kU/l, specific IgE guinea pig epithelia 78.6 kU/l (5), dog 3.71 (3), cat 0.87 (2), hamster <0.35. Daughter, positive skin tests to pollens, dander negative, total IgE 473 kU/l, specific guinea pig IgE > 100 KU/l (6), dog 1.95 kU/l, cat 1.73 kU/l, rest negative. Son, positive skin tests to pollens, dog 4 mm, total IgE 95 KU/l, guinea pig IgE 1.67 kU/l, dog 0.65 KU/l.

Conclusion: 1 We present three cases in a family of rhinoconjunctivitis, asthma and urticaria due to guinea pig epithelium sensitisation.

2 We highlight the importance of the guinea pig as allergenic source that gets to produce rhinoconjunctivitis-asthma cases in both homes and in laboratories.

3 In this case, it has been shown that the guinea pig was responsible for many cases of allergies and specified guinea pig IgE > 100 kU/l values. After removal of the animal from home and transfer to the next floor, symptoms begin to appear in the other family.

723

Predominant aeroallergens central Mexico City

Aguilar, D¹

¹Immunology, Hospital Juarez del Centro, Mexico, Mexico

Background: Mexico City is mainly horizontal, covers an area of 3500 km². Has a population of 21 million people with high population migration within the country. This makes a mosaic allergenic modifications are made to the environment where these groups are established. The city center features allergenic very particular about the lack of green areas, air pollution levels, the brutal deforestation and changes made to the environment by man. The Juarez Hospital Center is located in the city center, serving patients living in these areas so we selected patients with allergic rhinitis and / or allergic asthma diagnosed in the Allergy Department.

Method: We perform skin tests for immediate reading scarification with 40 pollen allergen extracts of grass and weeds, trees, mold spores and indoor-allergens previously selected by their frequency in this area and 15 min reading. We took as positive response + + + + and + + + and to record the results and use descriptive statistics. We employ 300 patients without separation of sex or age who using 1757 total tests.

Results: Ambrosia elatior 117 positive tests

Prosopis juliflora 95 positive tests

Quercus rubrum 87 positive tests

Mites 74 positive tests

Atriplex bracteosa 69 positive tests

Pleum pratense 68 positive tests

Artemisia vulgaris 66 positive tests

Fraxinus americana 59 positive tests

Taraxacum officinale 59 positive tests

Amaranthus palmeri 56 positive tests

Alternaria alternata 53 positive tests

Cynodon dactylon 53 positive tests

Ambrosia trifida 52 positive tests

Hellantus annus 52 positive tests

Penicillium notatum 50 positive tests

Conclusion: We found an increased frequency of wild herbs and grasses, interpreted by developing vacant lots and a large decrease in trees because of deforestation. Intense suffering our city for its inhabitants and reduced green areas.

724

Are rhinitis and asthma associated in elderly?

Pite, H¹; Morais-Almeida, M¹; Pereira, AM²; Todo-Bom, A³; Nunes, C⁴; Bousquet, J⁵; Fonseca, J⁶

¹Immunology Department, Hospital CUF Infante

Santo and Hospital CUF Descobertas, Lisbon, Portugal;

²Allergy and Clinical Immunology Department, Hospital S. João EPE, Porto, Portugal;

³Immunology Department, Hospitais da Universidade de Coimbra, Coimbra, Portugal;

⁴Centro de Imunoalergologia do Algarve, Portimão, Portugal;

⁵Department of Allergy and Respiratory Diseases, University Hospital and INSERM, Montpellier, France;

⁶CINTESIS – Center for Research in Health Technologies and Information Systems, Porto, Portugal

Background: Allergic rhinitis is a frequent chronic respiratory disease in both children and adults. It is considered an important risk factor for asthma. However, few data on rhinitis prevalence and its association with asthma is available in elderly populations. Thus, we aimed to assess the association between rhinitis and asthma in individuals with 65 years or older.

Method: Data was obtained from a cross-sectional, nationwide, population-based survey, accomplished through face-to-face questionnaire application to responders aged above 65 years old, in Portugal, who gave their informed consent. Current rhinitis was defined as the presence of at least two out of three rhinitis symptoms (repeated sneezing and/or itchy nose, blocked nose for more than 1 h or runny nose without having a cold or flu), either usually or in the last 12 months. Current rhinitis was further classified according to the ARIA recommendations. Asthma was defined by a positive answer to the question ‘Has a doctor ever said you have asthma?’. Univariate analysis was used to assess associations between current rhinitis and asthma and results were presented as odds ratio (OR) with 95% confidence interval (95%CI).

Results: Data was obtained from 3678 responders, 58.5% females, mean (standard deviation) age of 74.1 (7.0) years old. The prevalence of current rhinitis was 29.8% (95%CI 28.4–31.3%); no statistically significant differences were found for gender, age or urban/rural living area. Asthma was reported by 29.6% of subjects with current rhinitis. Current rhinitis had a strong association with asthma (OR 13.9, 95%CI 10.7–18.0). This association was evident in all rhinitis types: mild intermittent (OR 8.3, 95%CI 6.1–11.4), moderate-severe intermittent (OR 14.6, 95%CI 10.4–20.5), mild persistent (OR 16.5, 95%CI 9.7–28.0) and strongest in subjects with persistent moderate-severe rhinitis (OR 39.9, 95%CI 27.5–58.0).

Conclusion: Rhinitis is frequent in the elderly. Consistent with the results from earlier studies addressing different population age groups, we found a strong association between rhinitis and the presence of asthma, also in elderly. This reinforces the need to address upper airway symptoms in elderly population and to evaluate older patients with rhinitis for asthma, especially those with persistent and more severe rhinitis forms.

Conclusion: Rhinitis is frequent in the elderly. Consistent with the results from earlier studies addressing different population age groups, we found a strong association between rhinitis and the presence of asthma, also in elderly. This reinforces the need to address upper airway symptoms in elderly population and to evaluate older patients with rhinitis for asthma, especially those with persistent and more severe rhinitis forms.

Conclusion: Rhinitis is frequent in the elderly. Consistent with the results from earlier studies addressing different population age groups, we found a strong association between rhinitis and the presence of asthma, also in elderly. This reinforces the need to address upper airway symptoms in elderly population and to evaluate older patients with rhinitis for asthma, especially those with persistent and more severe rhinitis forms.

Conclusion: Rhinitis is frequent in the elderly. Consistent with the results from earlier studies addressing different population age groups, we found a strong association between rhinitis and the presence of asthma, also in elderly. This reinforces the need to address upper airway symptoms in elderly population and to evaluate older patients with rhinitis for asthma, especially those with persistent and more severe rhinitis forms.

725

The Canadian allergy, genes and environment network of centres of excellence: AllerGen

Denburg, J^{1,2}; Keith, PK^{1,3}

¹AllerGen NCE Inc, Hamilton, ON, Canada;

²Allergy & Immunology, McMaster University, Hamilton, ON, Canada;

³Medicine, McMaster University, Hamilton, ON, Canada

Background: AllerGen NCE Inc. is a national research network of Canada's leading experts in allergic diseases, asthma and anaphylaxis.

Methods: By collaborating with research institutions, multi-sectoral organizations and stakeholders, AllerGen works to address unmet biomedical needs; mobilise knowledge for new evidence-based public health policies and tools; discover new diagnostic tests and therapies; and, increase the number of allergy and clinical immunology professionals by providing international exchange opportunities to build capacity and promote multi-network interactions. AllerGen aims to improve the quality of life for allergy and asthma sufferers and their families, lessen the socio-economic

burden and reduce the risks of these diseases.

Results: AllerGen has completed its first 7 year mandate, and has been renewed for a second term, during which it will focus on 3 major Legacy Projects, already developed and launched: A national, multi-disciplinary birth cohort to follow the development of allergies and asthma from pregnancy to childhood and beyond, the Canadian Healthy Infant Longitudinal Development (CHILD) Study, which has recruited almost 4000 families; A multi-centred clinical trials consortium with international outreach, the Clinical Investigator Collaborative (CIC), in partnership with biopharmaceutical and biotechnology firms; and, a multi-centred, trans-disciplinary food allergy research consortium, The Canadian Food Allergy Strategic Team (Can-FAST). These three national initiatives are complemented by Enabling Platforms of research that AllerGen has established across Canada and internationally: Gene-Environment Interactions, working to apply genetic, environmental and epigenetic research innovation to discover novel therapies and diagnostics, and novel public health interventions and policies; Biomarkers and Bioinformatics, using standard operating protocols and animal models to develop a world-leading, systems-biology approach for development and commercialization; and, Patient, Policy and Public Health research, to provide patient and health professional outreach, and develop educational disease management tools.

Conclusion: AllerGen has become a leader in mobilizing research, catalyzing social innovation and enabling knowledge translation to help prevent, manage and treat allergy, asthma, and anaphylaxis. The model adopted by AllerGen is open to the global community to further develop allergy research and innovation.

728

What does the general population know about allergology?

Barasona Villarejo, MJ¹; Barasona Villarejo, ML²; García Nunez, I¹; Algaba Marmol, MA³; Guerra Pasadas, F¹

¹Allergology, Hospital Reina Sofía, Córdoba, Spain;

²Centro Universitario de Magisterio Sagrado Corazón, Córdoba, Spain; ³Hospital Reina Sofía, Córdoba, Spain

Background: A high percentage of the population presents symptoms compatible with allergy. The diverse forms of presentation of allergy and difficulty in accessing specialists led to set ourselves the task of finding out if the general population knows our specialist area and determining the

knowledge they have of general allergology concepts.

Method: Participants were asked to complete an anonymous questionnaire which asked about basic sociodemographics (age, sex, educational level, allergies, habitat), and different questions about the allergist's specialist field (general block, respiratory allergy block, food, hymenoptera, medicines, treatment and aeroallergens), using 44 questions with three options and only one correct answer. These were evaluated with +1 for the correct answer; incorrect -1 and 0 don't know/no answer.

Results: Three hundred people completed the questionnaire (116 males, 39%), with a mean age of 23 years. Ninety-nine (33%) considered themselves allergic and 100% of these had been seen by a doctor for this reason. Only 24 were undergoing immunotherapy. One hundred and ninety-two (64%) were university students. One hundred and twenty-one (40%) felt that the topic of allergies was quite interesting. The final average score was 13 points. Ninety-five had searched the Internet about health questions. Eighty-five percent knew who the allergist is, 28% associated the word spring with allergy even with high fever, 16% were unaware that someone with allergy to bee stings can die after being stung, 225 (75%) were unaware that alternaria is a fungus, only 10% knew that asthma can be cured by vaccination and 17% believed that people with allergy to olive trees cannot eat olives.

Conclusion: The general population has limited knowledge of allergology. The low percentage of correct answers to some questions suggests that we need to emphasize the importance of health education on increasingly prevalent diseases in the general population.

729

Use of epinephrine in a tertiary hospital

Ponce Guevara, V¹; Moreno Rodilla, E¹; González Ruiz, A¹; Muñoz-Bellido, F¹; Laffond Yges, E¹;

Dávila González, I¹

¹Allergology, Hospital de Salamanca, Salamanca, Spain

Background: Anaphylaxis is a serious allergic reaction of rapid onset that might cause death. Epinephrine is considered the treatment of choice. Nevertheless, the use of epinephrine for the treatment of anaphylaxis in the emergency departments (ED) is not well-known. We evaluate the use of epinephrine in a prospective study of patients that were assisted for an anaphylactic reaction in the ED of a tertiary hospital.

Method: Patients with anaphylaxis were identified reviewing the diagnosis of

patients that were assisted in the ED from September 2011 to August 2012. Initially, the medical records of patients discarded with any of the following diagnosis [International Classification of Disease, Ninth Revision (CIE 9)] were selected: Anaphylactic shock caused by food (995.60–995.69), other anaphylactic shock (995.0), angioneurotic edema (995.1), urticaria (708), allergic urticaria (708.0), idiopathic urticaria (708.1), an unspecified adverse effect caused by the correct administration of a drug, medicinal, and biologic substance (995.2) or an unspecified allergic reaction (995.3), other specified urticaria (708.8), unspecified urticaria (708.9), edema of larynx (478.6), edema of pharynx or nasopharynx (478.25) and the toxic effect of venom (989.5). All these medical records were revised to chose those compatible with anaphylaxis as proposed by the National Institute of Allergy and Infectious Disease and the Food Allergy and Anaphylaxis Network. For this study, data were collected on age, sex, and hypotension, use of epinephrine in ED and prescription of epinephrine auto-injector at discharge.

Results: During the follow-up period, 148 712 patients consulted the ED. A total of 89 (0.06%) patients fulfilled the NIAID/FAAN diagnostic criteria for anaphylaxis. Overall, 50.6% of the study sample was male. The median age of the study population was 44 years. From the global sample of patients only 41.6% received epinephrine. From the 29% of patients that had hypotension only 58% received epinephrine. Among patients discarded from the ED only 5.6% received a prescription for self-injectable epinephrine.

Conclusion: Although epinephrine is the treatment of choice of the anaphylactic shock, it was only administered to half of patients that had a severe anaphylaxis and the prescription of self-injectable epinephrine was extremely low.

731

The education program for parents of children with allergic diseases

Golysheva, E^{1,2}; Mokronosova, M^{1,2}

¹Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia; ²Allergology, Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia

Background: Children suffering from atopic dermatitis (AD) and bronchial asthma (BA) are known to develop itchy skin, respiratory disorders, disruptions of normal sleep patterns, psychological problems, such as irritability and restlessness. The reception of a patient usually lasts 20 min, thus giving the allergologist hardly any

time to educate the parents on skin care essentials and asthma monitoring. The above problem can be solved through education at schools for parents of children suffering from AD and BA.

Method: The education courses for 156 parents of children suffering from AD and BA were conducted in the 2011–2013 period. The courses included the following lectures: 'Skin Care for AD in Children', 'Dietary Therapy for Allergic Children', 'Asthma School', and 'The Road to Hypoallergenic Home'. The 40-min lectures were followed by question-answer sessions that lasted 20 min. The parents were asked to fill out the before and after-course questionnaires to identify any mistakes they could make in the care of children with allergy; the disease severity assessment and QLQ were offered before and after the course.

Results: The pre-course questionnaire-based survey has shown that all the parents made mistakes in their care of the allergic children. The main mistakes in the allergic disease management included the incorrect topical skin treatment, inhalation technique, application of antihistamines, administration of hypoallergenic diets, measures aimed to decrease the level of inhalant allergens in patients' homes. Most of the course students believed that the first generation antihistamines were safer than those of the second generation, that topical steroids were harmful to children, that non-traditional therapy methods were more effective than the conventional ones, that they could find 'a hypoallergenic cat or a dog'. Some of them had psychological problems caused by their fear of a severe allergic reaction to a particular food or medication. They didn't know how to behave in emergency situations. The questionnaires completed 6 months after the education course have shown positive changes: The parents corrected their previous mistakes regarding the allergic child care. The monitoring has demonstrated a decrease in the disease symptom severity score and an improvement in QL of 127 children.

Conclusion: Educational courses conducted at schools for the parents of children with allergy are an essential component of allergy practices.

732

National project for community based atopy asthma friendly school in Korea

Chung, EH^{1,2}; Seo, H-J²; Jou, H-M²; Suh, S-H³; Jeong, J-Y⁴; Lim, YS²; Ghim, Y-A⁵; Kim, YT³; Park, HK²

¹National Medical Center, Pediatrics, Seoul, Korea;

²Division of Chronic Disease Surveillance, Korea Centers for Disease Control and Prevention, Osong, Korea;

³Center of Infection Disease Control, Korea Centers for Disease Control and Prevention, Osong, Korea; ⁴Korea Health Promotion Foundation, Seoul, Korea; ⁵Division of Health and Nutrition Survey, Korea Centers for Disease Control and Prevention, Osong, Korea

Background: In Korea, there has been an increase in the prevalence of allergic disease among the children and adolescents. Attention has focused on the system to monitor and manage the current situation. Schools are an attractive and popular setting for implementing interventions for adolescents. Therefore, the government has planned the pilot project for the prevention of atopy and asthma.

Method: The schools, kindergarten and day-care center interested in this project were selected. Principals and school health nurses (or child care teachers) in this schools were invited to register workshops informing specifics regarding the operation of Atopy Asthma Friendly School. School health nurses were taught the way to deal with emergency situation related to asthma and to make use of emergency kit (Ventolin, Spacer, Emergent treatment leaflets and Moisturizers). Students with atopy or asthma were selected and thoroughly monitored by trained teachers. The patients and parents participated in counseling sessions on the prevention, and treatment of allergic disease. Data were collected with questionnaires on 166 school health nurses working at Atopy Asthma Friendly School.

Results: From 2008 to 2012, participating schools were 18, 144, 238, 355, and 474 respectively. In 2012, the number of students was raised up to 156 870 from 56 011 in 2009. The prevalence rates of asthma in these schools were 7.0%, 7.3%, and 7.4% of investigated students for 2008, 2009, and 2010 respectively. The prevalence rates of allergic rhinitis were 28.0%, 29.3%, and 31.7% of investigated students for 2008, 2009, and 2010 respectively. The prevalence rates of atopic dermatitis were 28.2%, 28.3%, and 26.3% of investigated students for 2008, 2009, and 2010 respectively. In 2011, emergency kits were provided to 266 Atopy Asthma Friendly Schools, 29 schools (10.9%) had used Ventolin at least once. Ninety-two percent of 166 school health nurses working at Atopy Asthma Friendly Schools said that this project was necessary. The questionnaires showed that they needed education programs for students and guardians (28.9%), establishment

of hospital linked system (23.9%), education on disease (21.1%), and manual on emergency situation (19.6%).

Conclusion: Atopy Asthma Friendly School provides a model for addressing the asthma and atopy-related problems among children and adolescents. Atopy Asthma Friendly School was effective in improving school life and overall health status of children and adolescents.

734

Evaluation of allergic diseases among students of university in Moscow

Kozulina, I¹; Kurbacheva, OM¹; Pavlova, KS¹

¹Institute of Immunology of the Federal Medicobiological Agency, Moscow, Russia

Background: Allergy represent global problems for all age groups. About 30% of all adult population and about 50% of children has different types of allergy: allergic rhinitis, atopic asthma, atopic dermatitis, food and insect allergy etc. The aim of this study was to analyze the epidemiology situation in group of young people (birth 1990–1995) in Moscow.

Method: 350 students of university (212 males and 138 females, birth 1990–1995) were surveyed with the help of random sampling using the 'Questionnaire to identify the structure and distribution of allergy disease amount residents of Moscow'.

Results: Thirty-two males (15%) and 11 females (7%) have atopic asthma, which was diagnosed in 3–15 years old. 35 of them (81%) take asthma therapy. In 56 males (26%) and 46 females (33%) reported symptoms of rhinitis and conjunctivitis during the period of flowering, during cleaning the house and in contact with the animals, and 35 of them all (34%) do not pass a particular allergodiagnostic. In addition 10 cases in male group and in six cases in female group we can suggest an atopic disease. In 40 males (18%) and 38 females (27%) has symptoms of food intolerance, 26 males (12%) and 24 females (17%) has symptoms of drug intolerance, especially for antibiotics. In 39 respondents (22%) in each gender group has allergic skin symptoms: 17%- atopic dermatitis, 35%- symptoms of no diagnostic urticaria. In 70 (20%) of all respondents has a genetic predisposition in atopic diseases.

Conclusion: The incidence of allergy among young people is high. Despite increasing incidents, the rate of timely diagnoses and detection of allergy is low. To improve diagnosis and early treatment of patients with different forms of allergy should be more careful approach to the diagnosis and identification of patients with allergy.

Poster Session 20

Pediatric allergy and clinical immunology

735

Four year experience of national campaign for the diagnosis and prevention of allergic skin diseases in Bulgaria

Darlenski, R¹; Gergovska, M²; Yankova, R³; Hristakieva, E⁴; Kadurina, M⁵; Tonev, S⁶; Gospodinov, D⁶; Demerdjieva, Z¹; Nikolova, A²; Brambarova, D³; Tsankova, L⁵; Grozeva, D⁶; Gancheva, D⁴; Todeva, V⁴; Gospodinova, K⁶; Tsankov, N¹; Kazandjieva, J²; Dermatoallergy Section of the Bulgarian Dermatological Society
¹Dermatology and Venereology, Tokuda Hospital Sofia, Sofia, Bulgaria; ²Dermatology and Venereology, Medical University-Sofia, Sofia, Bulgaria; ³Dermatology and Venereology, Medical University Plovdiv, Plovdiv, Bulgaria; ⁴Medical Faculty, Dermatology and Venereology, Trakia University-Stara Zagora, Stara Zagora, Bulgaria; ⁵Dermatology, Venereology and Allergology, Medical Military Academy Sofia, Sofia, Bulgaria; ⁶Medical Faculty, Dermatology and Venereology, Medical University-Pleven, Pleven, Bulgaria

Background: Allergy is considered as one of the epidemics of the XXI Century. The prevalence of the most allergic diseases is increasing in the past decades. Skin accomplishes the contact with the surrounding environment and is often affected by allergic diseases. Herein we present the introduction and the four-year-experience of the Bulgarian Dermatological Society in organizing National campaign for the diagnosis and prevention of allergic skin diseases in Bulgaria.

Method: Free of charge dermatologic consultation and skin allergy testing were provided in 6 university hospitals and dermatology clinics in the biggest cities. The doors were open for 5 days, each November. In addition, a press conference aiming to attract the community's attention on the social and economical impact on skin allergy was organized each year.

Results: The summary of the results showed an increase in the number of the consulted patients starting 420 people in 2009 and exceeding 500 in 2012. *In vivo* skin testing was performed in 220 patients in average, the majority of them patch testing with European baseline series. The analysis of the results showed that nickel was the most common contact allergen each year followed by other metals, PPD, preservatives and perfume ingredients.

Conclusion: Sharing our experience in organizing the campaign and discussing the strong and weak points is a safe ground

for developing such activities in other countries.

736

Prevalence of eczema and parent reported triggers in early and late childhood

Venter, C^{1,2}; Patil, VK^{1,3}; Glasbey, G¹; Grundy, J¹; Dean, T^{1,2}; Arshad, SH^{1,3}
¹David Hide Asthma & Allergy Research Centre, Newport, United Kingdom; ²University of Portsmouth, Portsmouth, United Kingdom; ³University of Southampton, Southampton, United Kingdom

Background: Eczema is a common allergic condition in childhood. We investigated the prevalence of reported and diagnosed eczema and also parent reported causes of eczema in early childhood and later childhood.

Methods: The FAIR (Food Allergy and Intolerance Research) birth cohort (*N* = 969) was established on the Isle of Wight (UK) between September 2001 and August 2002 to prospectively study the natural history of food allergy. Participants were followed up at 1, 2, 3 and 10 years of age. We looked at the prevalence of reported and physician diagnosed at 1 and 10 years. For the children with physician diagnosed eczema, we explored parent reported triggers (defined by 'Have you identified a cause for the eczema?'). Outcomes of 1 year and 10 year follow up were analysed.

Results: At 1 year 898/969 (92.7%) and at 10 years 826/969 (85.2%) children were followed up and parents completed a questionnaire which included the above questions on eczema. At 1 year 225/898 (25.1%) parents reported eczema in their child and 168/898 (18.7%) reported physician diagnosed eczema irrespective of reported symptoms. Of the 225 who reported eczema, 165 (73.3%) were diagnosed by a physician. Of the 168 with diagnosed eczema, 32 (19%) parents reported to have identified a cause; food was identified in 8 (25%), (6 milk and 2 egg), 6 (18.8%) said soap or washing powder as cause, 12 (37.5%) identified 'teething' as cause and the remaining parents reported other causes. At 10 years 254/826 (30.8%) parents reported eczema in the children and 258/826 (31.2%) reported their child

to have been diagnosed with eczema in the first 10 years of life. Of the 254 who reported symptoms at 10 years, 210 (82.7%) were diagnosed with eczema. Parents of 74/258 (29.2%) children with diagnosed eczema had identified a cause; aeroallergen by 17 (23.2%), food allergen by 3 (4%), weather or temperature in 19 (25.7%), soap (shower/bath) or washing powder in 12 (16.2%), central heating in 4 (5.4%) and stress in 2 (2.7%).

Conclusion: In the first year of life a quarter of parents reported eczema symptoms and three quarters of those had a physician's diagnosis of eczema. At 10 years of age almost a third of parents reported eczema symptoms and 82% have been diagnosed with eczema. Triggers reported by parents varied between early and late childhood; food, teething and soap/washing being the common ones at 1 year and weather/temperature, aeroallergen and soap/washing being common at 10 years of age.

737

Characteristics of phenotypic clusters in atopic dermatitis from population based school-aged cohort

Kwon, JW¹; Kim, YH¹; Jung, YH²; Lee, E²; Yang, SI²; Kim, HY²; Seo, JH³; Kim, BJ⁴; Kim, HB⁵; Lee, SY⁶; Yu, J²; Kwon, HJ⁷; Hong, SJ²

¹Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Korea; ²Department of Pediatrics, Childhood Asthma Atopy Center, Research Center for Standardization of Allergic Diseases, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; ³Department of Pediatrics, Korea Cancer Center Hospital, Seoul, Korea; ⁴Department of Pediatrics, Inje University Haeundae Paik Hospital, Busan, Korea; ⁵Department of Pediatrics, Inje University Sanggye Paik Hospital, Seoul, Korea; ⁶Department of Pediatrics, Hallym University Seced Heart Hospital, Suwon, Korea; ⁷Department of Preventive Medicine, College of Medicine, Dankook University, Cheonan, Korea

Background: Atopic dermatitis is a pathophysiological heterogeneous disease composed by several clinical phenotypes sharing common clinical features. Unbiased multidimensional methods to classify phenotypes of atopic dermatitis have a possibility for individualised approach. We evaluate the characteristics of atopic dermatitis in general population based primary School children cohort using cluster analysis.

Method: A TwoStep cluster analysis was performed in 461 subjects who had a current atopic dermatitis using eight important variables (gender, body mass index, history of asthma, history of allergic rhinitis, parental history of atopic dermatitis, atopic sensitisation, log total IgE, and blood eosinophil%) affecting to childhood atopic dermatitis. We compared differences in demographic and biologic characteristics between clusters.

Result: Four clusters were extracted from 425 children with atopic dermatitis after excluding missing values. The first cluster ($n = 99$) was non-atopic female group (0% atopy) with low total serum IgE (log IgE = 1.90 ± 0.53 , $P < 0.001$) and relatively late onset (78.3% after 12 months of age). The second cluster ($n = 158$) was associated with higher rate of parental history of allergic diseases (55.7%, $P < 0.001$) and combined history of allergic rhinitis (100%, $P < 0.001$) and asthma symptom (24.7%, $P = 0.009$). The third cluster ($n = 87$) was comprised of atopic eczema (100% atopy) with higher level of bronchial hyperresponsiveness (log PC₂₀ = 1.12 ± 0.64 , $P = 0.002$) and serum total IgE (log IgE = 2.35 ± 0.52 , $P < 0.001$), and multiple sensitisations (57.5%, $P < 0.001$). The forth cluster ($n = 81$), non-atopic male group, was associated with lower level of total IgE and bronchial hyperresponsiveness. Use of antibiotics in infancy was associated with the second cluster (51.7%, $P < 0.001$), and the exposure to environmental tobacco smoke with the third and the forth cluster (59.3%, 59.0%, $P = 0.046$).

Conclusion: Atopy, gender, and the history of respiratory allergic disease are important factors to determine the subtypes of current atopic dermatitis in childhood. Some environmental factors are associated with specific phenotypes of atopic dermatitis.

This study was supported by a grant of Ministry of Environment, Republic of Korea. 'Children's Health and Environmental Research'.

738

Time changes in eczema and environmental risk factors in Skopje, The Republic of Macedonia

Vlaski, E¹; Stavric, K²; Kimovska, M³; Seckova, L¹; Kirovski, I¹

¹Department of Pulmonology and Allergology, University Children's Clinic, Skopje, FYR Macedonia;

²Department of Immunology, University Children's Clinic, Skopje, FYR Macedonia; ³University Children's Clinic, Intensive Care Unit, Skopje, FYR Macedonia

Background: Decreased or leveled eczema symptom prevalence in young adolescents in some developed countries with previously high prevalence rates and further

increase in formerly low-prevalence developing countries have been recently reported. The role of environment and its changes seem to be important. The aim of the study was to assess the 4-year time changes in eczema and some environmental risk factors among young adolescents in Skopje, the capital of the Republic of Macedonia as a developing country with low prevalence of eczema.

Method: Self-reported data obtained through ISAAC phase 3 written questionnaires by 3026 13–14-year-old adolescents in 2001–2004 survey and by 1088 same-aged adolescents in 2005–2008 survey from randomly selected primary schools in Skopje was analysed. Chi-square test was performed to test for statistical significance in comparisons related to eczema and environmental factors prevalence rates between the two surveys and results were expressed as odds ratios (OR, 95%CI) in logistic regression.

Results: In 2005–2008 survey, compared to 2001–2004 survey, significantly higher prevalence of severe eczema symptoms (2.5% vs. 1.5%, $P = 0.03$) and non-significantly lower prevalence rates of eczema symptoms and ever-diagnosed eczema were established. Adolescents in 2005–2008, compared to 2001–2004 survey, reported higher intake of fish (6.2% vs. 3.6%; 1.90, 95%CI 1.38–2.62), vegetables (70.9% vs. 55.6%; 1.95, 1.68–2.27), rice (9.2% vs. 6.1%; 1.54, 1.19–1.99) and nuts (16.8% vs. 13.1%; 1.34, 1.10–1.62), and lower intake of milk (62.7% vs. 68.3%, 0.78, 0.67–0.90), margarine (24.9% vs. 28.9%; 0.82, 0.69–0.96), eggs (20.3% vs. 23.6%, 0.83, 0.69–0.98), cat and dog ownership (13.5% vs. 24.1%; 0.49, 0.41–0.60 and 22.0% vs. 27.7%; 0.74, 0.62–0.87) and wood home heating (10.8% vs. 18.0%; 0.55, 0.45–0.68), respectively.

Conclusion: The two cross-sectional surveys 4-year apart showed slight decrease in eczema symptoms and diagnosis, and increase in severe eczema symptoms. Changes in lifestyle such as diet, pets ownership and indoor airpollution as well rapid westernization in Macedonia, as a developing country, may have some contribution to the time changes in eczema.

739

Moscow newborns-2011: prevalence of allergies in families and the outcome

Trenea, M¹; Munblit, D²; Ivannikov, NY³; Pampura, A¹

¹Allergy Department, Moscow Institute of Paediatrics and Child Surgery, Moscow, Russia; ²Imperial College London, Paediatrics, London, United Kingdom;

³Moscow No.1 Maternity Hospital, Moscow, Russia

Background: Knowledge of the family allergy history is of an extreme importance

for the primary prevention of allergic diseases in children. Self-reported parents and siblings allergy symptoms can be valuable for the future allergy diagnosis of a newborn child and are useful for allergy prophylaxis.

Objective: To evaluate the prevalence of allergic symptoms in parents and siblings of newborn children and to evaluate potential targets for the primary allergy prevention.

Methods: Thirteen visits to the Postnatal Department of Moscow No.1 Maternity Hospital were carried out in Oct-Dec 2011. Women delivered within the preceding 48 hours were interviewed. Mothers provided research team member with information on relatives of a newborn in regards to clinical manifestations of food allergies, atopic dermatitis, eczema, urticaria, angioedema, allergic rhinitis, allergic conjunctivitis, asthma and episodes of drug allergy. Information on parents and siblings of 393 newborns was collected.

Results: Mothers of Moscow newborns-2011 have self-reported allergies in 24.94% (95%CI 20.25...29.62), fathers in 16.03% (95%CI 12.02...20.05), sisters ($n = 100$) in 30.00% (95%CI 19.68...40.32), brothers ($n = 120$) in 31.67% (95%CI 22.20...41.13). Allergy primary prevention group in newborns is 16–32% if one close relative have allergies and 14.5% if two close relatives have allergies.

Conclusion: More than 15% of families of Moscow newborns-2011 have allergies. Prevalence of allergy in siblings exceeds those in parents. A group for primary prevention of allergy is about 15–30% of newborns.

740

Prevalence of allergic diseases based on medical claim data from National Health Insurance Corporation in Korea

Lim, DH^{1,2}; Seong, HU³; Kim, JH¹; Son, BK¹

¹Inha University, College of Medicine, Incheon, Korea;

²Environmental Health Center, Ministry of Environment, Inha University Hospital, Incheon, Korea; ³Inha University Hospital, Incheon, Korea

Background: It is widely known that allergic diseases progress through a sequential course known as the allergic march. However, there have been no recent reports in Korea regarding the progress of allergic diseases based on the medical claim data of the National Health Insurance Corporation.

Method: Medical claim data of 2005 and 2008 from the National Health Insurance Corporation were used. Data was classified according to the administrative districts of metropolitan cities and provinces, and

divided according to age in increments of 5 years.

Results: The mean prevalence of allergic rhinitis is 7.79% in 2005 and 9.08% in 2008. The prevalence of atopic dermatitis is 2.33% in 2005 and 2.18% in 2008. The prevalence of asthma is 4.61% in 2005 and 4.57% in 2008. Figure 1 shows the pattern of change of prevalence of allergic diseases (allergic rhinitis, atopic dermatitis, and asthma) according to the age.

Conclusion: According to the nationwide survey on the prevalence of allergic diseases using the medical claim data from the National Health Insurance Corporation, the prevalence of allergic rhinitis had significantly increased in 2008 compared to 2005. More survey studies should be conducted in the future using the medical claim data of the National Health Insurance Corporation.

741

Influence of environmental and infectious factors on difference of prevalence of allergy in urban and rural children in Guangdong, China

Feng, M; Pan, L; Yan, C; Li, J
State Key Laboratory of Respiratory Disease,
Department of Allergy and Clinical Immunology,
The First Affiliated Hospital of Guangzhou Medical
College, Guangzhou, China

Background: Higher rates of asthma and allergen sensitisations have been reported in urban than in rural areas. The purpose of this study was to investigate the impact of environmental and infectious factors on the prevalence of asthma in urban and rural children in Guangdong province of China

Method: Three hundred and seventeen children (188 from urban Guangzhou, 129 from rural Conghua) with self-reported asthma symptoms and 537 healthy subjects were selected from schools according to the ISAAC Phase I screening questionnaires. All subjects were completed a detailed questionnaire and medical examinations including skin prick test to 8 common aeroallergens, lung function test, histamine bronchial provocation test, peripheral blood for hepatitis A virus antibody and salmonella antibody ELISA. House dust samples were collected from living room and mattresses of 156 of these children (Guangzhou 76, Conghua 80) for detection of endotoxin and allergen.

Results: The prevalence rate of wheeze in Guangzhou and Conghua schoolchildren were 6.9% and 3.4% respectively by screening questionnaire ($P < 0.001$). The prevalence of wheeze symptom, asthma, nasal symptoms and allergic rhinitis in the children of Guangzhou were significantly

higher (1.72%, 1.66%, 3.97%, 2.77%, respectively) than those of Conghua (0.38%, 0.19%, 2.31%, 0.46%, respectively, $P < 0.01$) according to detail questionnaires. Endotoxin concentrations in dust samples in homes of Conghua were significantly higher (1794 EU per square meter, 10.95 EU endotoxin/g dust) than those of Guangzhou (508.8 EU per square meter, 6.45 EU endotoxin/g dust, $P < 0.01$). Logistic regression analyses showed a positive association between endotoxin levels and PD20-FEV1 ($r = 0.174$, $P < 0.05$), and a negative association ($P < 0.01$) between endotoxin levels and the wheal sizes of skin response to *Dermatophagoides pteronyssinus* (Der. p) and *Dermatophagoides farinae* (Der. f). The univariate logistic regression analysis showed a positive association between HAV-antibody and nasal symptoms. Salmonella antibody positive rate in children in Guangzhou was significantly higher than those in Conghua (29.2% and 21.9%, respectively, $P < 0.05$).

Conclusion: Exposure to environmental endotoxin might contribute to the lower risk of allergic sensitisation, and the previous infectious history may influence the development of allergic diseases.

The study is supported by Chinese National Science Funding (NSFC-AF-DFG 81261130023).

742

Autumn pollen induced allergic rhinitis and seasonal asthma: the progression and potential implications of serum specific IgE levels

Cui, L¹; Yin, J¹
¹Allergy, Peking Union Medical College Hospital,
Chinese Academy of Medical Sciences & Peking Union
Medical College, Beijing, China

Background: A close relation between rhinitis and asthma has been well documented. However, the coexistence of autumn pollen induced allergic rhinitis and seasonal asthma has drawn scant attention. We used cross-sectional data and later the follow-up data to investigate the frequency of seasonal asthma in patients with autumn pollen induced allergic rhinitis and the implications of specific IgE levels for asthma onset.

Method: 1085 patients with fall allergies were face-to-face interviewed from 2001 to 2003 and underwent serum specific IgE tests to inhaled allergens. The temporal sequence of allergic rhinitis and asthma was documented. In 2011 a follow-up study of 450 former participants without asthma at baseline was done by telephone to investigate asthma developing between the two surveys. Multinomial logistic

regression was used to examine the effect of specific IgE levels on the onset of asthma in patients with autumn pollen induced allergic rhinitis.

Results: By the end of the follow-up, there were 56 new cases of asthma. Of the 1028 participants with a positive IgE test to either *Artemisia* or *Humulus* and complete data for allergic rhinitis and asthma, 619 (57.1%) had a history of allergic rhinitis which preceded or occurred at the same time as asthma. 394 (36.3%) had allergic rhinitis only. The most majority of participants presented with allergic rhinitis and asthma only during the autumn pollen season. After controlling for sex, age at onset of allergic rhinitis, parental history of asthma, IgE levels 3–6 to *Humulus* or IgE levels 5–6 to *Artemisia* was associated with a higher risk of asthma onset at the same time as allergic rhinitis ($P < 0.05$). Similarly, allergic rhinitis with IgE levels 3–6 to *Humulus* or IgE levels 4–6 to *Artemisia* was at increased risk for asthma developing ($P < 0.05$).

Conclusion: Autumn pollen induced allergic rhinitis often coexist with seasonal asthma. The probability of asthma increased with increasing specific IgE levels.

743

Atopy prevalence and incidence in a cohort of children with recurrent wheezing: from pre-school age to adolescence

Pereira, AM^{1,2,3}; Gaspar, A²; Morais-Almeida, M²
¹Allergy Department, Centro Hospitalar São João,
E.P.E., Porto, Portugal; ²Immunology Department,
Hospital CUF-Descobertas, Lisbon, Portugal;
³Department of Health Information and Decision
Sciences, Faculty of Medicine of the University of
Porto, Porto, Portugal

Background: The aims of this study were to estimate baseline atopy prevalence in a cohort of pre-school children with recurrent wheezing (RW), to assess personal and socio-demographic factors associated with atopy in wheezers, and to estimate atopy incidence over a 13 years follow-up period.

Methods: This cohort study included 308 children observed as 1st appointments in a tertiary hospital's outpatient clinic in 1993. All children aged <7 years old and with RW (≥ 3 wheezing episodes responsive to bronchodilator in the previous year with symptom-free intervals in-between) were included. They were evaluated in an allergy consultation and skin-prick tests (SPT) to airborne allergens, including mites (Dp, Df), pollens (grasses, *parietaria*), moulds and pets (cat, dog), were performed. Atopy was defined as having at least one positive

SPT. The included children were reassessed in 2001 ($n = 249$; 225 with SPT) and 2006 ($n = 170$; 39 with SPT). A multiple logistic regression model was developed to study factors associated with atopy at pre-school age; results were presented as odds ratio (OR) with 95% confidence interval (CI).

Results: In 1993, the prevalence of atopy was 47% (95% CI[41–53]); 99% ($n = 143$) of the atopic wheezers had positive SPT to mites while only 8% ($n = 12$) were sensitised to pollens. Atopy at pre-school age was positively associated with rhinitis diagnosis (18.7[8.6–41.0]), wheezing beginning at ≥ 48 months of age (7.9[2.0–30.9]) and paternal history of asthma/rhinitis (2.7[1.2–6.5]); having recurrent respiratory infections (0.3[0.1–0.7]) and a pet at home in the 1st year of life (0.5[0.2–0.9]) presented a negative association. Maternal history of asthma/rhinitis and exposure to maternal tobacco smoke during pregnancy were not significantly associated with atopy. When considering the period 1993–2001, 60 children (out of 119 previously nonatopic that were tested in 2001) had *de novo* atopy diagnosis (incidence 50%). In the same period, the incidence of sensitisations to pollens was 27% (58/217) and to mites it was 46% (55/121). In 2001–2006, only 2 children had new atopy diagnosis (out of 9; incidence 22%).

Conclusion: The prevalence of atopy in pre-school children with recurrent wheezing was high and mites were the most prevalent sensitisers. Having a pet in the 1st year of life and presenting recurrent respiratory infections were negatively associated with atopy. Half of the wheezers without atopy at pre-school had *de novo* sensitisations at school age.

744

Prevalence of seasonal allergic rhinitis in schoolchildren in the years 1995, 2001, 2006 and 2011

Emeryk, A¹; Chojna, E¹; Kowalska, M¹; Markut-Miotla, E¹; Bartkowiak-Emeryk, M²; Postepski, J¹

¹Department of Lung Diseases and Rheumatology, University of Medicine in Lublin, Lublin, Poland;

²Department of Clinical Immunology, University of Medicine in Lublin, Lublin, Poland

Background: Allergic rhinitis (AR) is a common disease in children and adults worldwide. The increasing prevalence of

AR in children have been reported from different part of the world in the recent decades. The aim of our study was to estimate the prevalence of seasonal AR (SAR) in schoolchildren from urban region in Eastern Poland (Lublin) in 2011 and to compare the results with those of 2006, 2001 and of 1995.

Method: A repeated cross-sectional epidemiological study was performed in 1995 ($n = 599$), 2001 ($n = 565$), 2006 ($n = 548$) and 2011($n = 465$) in the population of children aged 6–13 randomly selected from the same primary school in Lublin, and with the use of the same written questionnaire. The questionnaire was based on the European Community Respiratory Health Survey (ECRHS) questionnaire (Emeryk A et al. *Ann Agric Environ Med* 2004;11:63–66). SAR was defined as: lifetime typical seasonal symptoms of AR plus positive skin prick test results with grass or/and trees or/and weeds pollen allergens. Diagnosis was confirmed by allergologist or pneumonologist. The response rate was 81.0%, 71.2%, 73.1% and 78.9% in 1995, 2001, 2006 and 2011, respectively. The cumulative prevalence of SAR was estimated.

Results: The principal results of the study are presented in the Table. Cumulative prevalence of SAR increased significantly from 5.5% in 1995 to 13.6% in 2001 ($P < 0.0001$). Although we observed the global trend of high prevalence in years 2001, 2006 and 2011 there were no statistical differences in the prevalence between these years (13.6 vs. 14.2% vs.15.9% respectively) ($P > 0.05$).

Conclusion: The prevalence of SAR has been estimated at high levels since 2001 but no significant changes in prevalence have been observed since then.

745

Prevalence and ARIA classification of rhinitis in pre-school children in Portugal: a nationwide cross-sectional study

Santos, N^{1,2}; Pereira, AM^{1,2,3}; Branco Ferreira, M^{4,5}; Nunes, C^{4,6}; Fonseca, JA^{1,7,8}; Bousquet, J⁹; Morais-Almeida, M^{2,4}

¹Allergy Department, Centro Hospitalar São João, E.P.E., Porto, Portugal; ²Immunology Department, Hospital CUF-Descobertas, Lisbon, Portugal;

³Department of Health Information and Decision Sciences, Faculty of Medicine of the University of Porto, Porto, Portugal; ⁴Sociedade Portuguesa de Alergologia e Imunologia Clínica, SPAIC, Lisbon, Portugal; ⁵Immunology Department, Centro Hospitalar Lisboa Norte, Lisbon, Portugal; ⁶Centro de Imunoalergologia do Algarve, Centro de Imunoalergologia do Algarve, Portimão, Portugal; ⁷Allergy Unit, Instituto and Hospital CUF Porto, Porto, Portugal; ⁸Faculty of Medicine of the University of Porto, CINTESIS – Centre for Research in Health Technologies and Information Systems, Porto, Portugal; ⁹Department of Allergy and Respiratory Diseases, University Hospital and INSERM, Montpellier, France

Background: Information concerning the epidemiology of rhinitis in young children is scarce. The aims of this study were to estimate the prevalence of current rhinitis in preschool children using the ISAAC definition, to describe individual rhinitis symptoms, to classify rhinitis according to the ARIA recommendations and to report socio-demographic characteristics associated with rhinitis in this age group.

Methods: Cross-sectional, nationwide, population-based study including children aged 3 to 5 years. Data was collected by face-to-face interview to caregivers using an adaptation of the ISAAC questionnaire. Current rhinitis was defined as the presence of at least one out of three rhinitis symptoms (repeated sneezing and/or itchy nose, blocked nose for longer than 1 h or runny nose without having a cold or flu), in the last 12 months; children with symptoms lasting at least 4 days/week and more than 4 consecutive weeks were considered to have persistent rhinitis. Mild rhinitis interfered ‘not at all’ or ‘a little’ in the child’s daily activities; moderate-severe disease interfered ‘a moderate amount’ or ‘a lot’

Results: Overall, 5018 children were included. The prevalence of current rhinitis was 43.5% [95%CI (42.1–44.9%)] and that of physician-diagnosed rhinitis was 11.7% [95%CI (10.8–12.6%)]. Of children with current rhinitis, 67% reported blocked nose, 48% rhinorrhea and 46% sneezing/itchy nose; 24% had symptoms with moderate to severe impact in daily routine. Considering the ARIA recommendations, 70% had mild intermittent disease, 6% mild persistent, 17% moderate-severe intermittent and 7% moderate-severe persistent.

Children living in a rural region had a significantly lower prevalence of ‘current rhinitis’ (36% vs. 46% in those living in

Table 1: The prevalence of SAR in schoolchildren in years

	Prevalence (%)			
	1995 ($n = 599$)	2001 ($n = 565$)	2006 ($n = 550$)	2011 ($n = 465$)
Allergic rhinitis				
SAR	5.5	13.6	14.2	15.9

urban regions, $P < 0.001$). Children with current rhinitis had more current wheezing (39% vs. 14% of those without rhinitis, $P < 0.001$), physician-diagnosed asthma (8% vs. 2%, respectively, $P < 0.001$), self-reported food allergy (8% vs. 6%, respectively, $P = 0.003$) and family history of allergic disease (35% vs. 24%, respectively, $P < 0.001$). These characteristics were also associated with more persistent and severe forms of rhinitis.

Conclusion: Rhinitis is a common but frequently underdiagnosed disease in pre-school children. This was the first epidemiological survey classifying rhinitis according to ARIA guidelines in this age group. About one-fourth of the children with current rhinitis presented moderate-severe disease.

746

Trends of prevalence of asthma and allergy in the western part of Georgia

Abramidze, T¹; Gotua, M¹; Lomidze, N¹; Mgaloblishvili, N¹; Kulumbegov, B¹; Gankrelidze, A¹

¹Center for Allergy and Immunology Research, Tbilisi, Georgia

Background: Given the considerable burden of asthma and allergy on children worldwide the prevalence studies that characterise these chronic disorders are invaluable. In 2003 and 2012, we performed cross-sectional studies in Kutaisi (western part of Georgia) schoolchildren aged 6–7 and 13–14 year., using the validated ISAAC methodology, aiming to provide the prevalence trends of asthma and allergy in this population.

Method: Both cross-sectional studies were carried out in the same city, same season and used identical methodologies. In 2003, number of participants in 6–7 and 13–14 years old groups were 2666 (RR- 92.8%) and 2650 (RR- 90.2%) correspondently. In 2012, number of participants in 6–7 and 13–14 years old groups were 3039 (RR- 84.7%) and 2339 (RR- 72.4%) correspondently.

Results:

Conclusion: The prevalence of asthma and allergies markedly increase among both age groups of schoolchildren in the western part of Georgia. The exact reasons for such trends remain to be explored.

747

Early sensitisation to dog and cat in a cohort of food allergic infants

Sugimoto, M¹; Nagao, M¹; Tanaka, A²; Önell, A²; Borres, MP²; Fujisawa, T¹

¹Institute for Clinical Research, Mie National Hospital, Tsu, Japan; ²ImmunoDiagnostics, Phadia AB, ThermoFisher Scientific, Uppsala, Sweden

Background: Prevalence of sensitisation to pets increases with increasing exposure. On the contrary, recent studies suggest that early perinatal exposure to pets protects from pet sensitisation/allergy later in life. In addition, it has been shown that the prevalence of sensitisation is increasing, the number of multisensitised patients are increasing and the onset of sensitisation tend to start earlier in life than previously observed.

Objectives: To describe the prevalence of pet sensitisation in young children and to explore the relationship between pet sensitisation and natural history of allergic disease.

Methods: The IRAM (Impact of Allergic Rhinitis on Atopic March in Children) cohort (UMIN000004157) from Japan enrolling 305 children <2 year of age with diagnosed food allergy and/or atopic dermatitis, and without diagnosed asthma, was studied. Physician-diagnosed asthma, symptoms of allergic rhinitis, histological analysis of nasal mucosa, and specific IgE (ImmunoCAP) to common food and airborne allergens were prospectively studied for 2 years. Interim results of sensitisation to dog/cat at entry (13.7 months old) and at 1 year for 221 children are presented here.

Results: Of the 221 children 72% were diagnosed with atopic dermatitis and 89% with food allergy. In addition, 80%, 45% and 27% of the cohort avoided egg, milk

and wheat based on doctors' diagnosis, respectively. Pet ownership (any) was reported for 12% of the children, and 10% kept dogs, while only 1% kept cats in their household. The prevalence of sensitisation (defined as specific IgE level >0.34 kU/l) to dog dander was 42% and 48%, and to cat dander 22% and 27% at entry and at 1 year follow-up, respectively. Sensitisation to house dust mite (HDM), the most prevalent inhalant allergen in Japan, was found in 36% and 63% of the subjects at entry and 1 year. The correlation of IgE to dog dander and milk was stronger than for IgE to dog dander and HDM, with Spearman's correlation coefficients of 0.52 and 0.32, respectively.

Conclusions: We found a surprisingly high prevalence of dog and cat sensitisation in this atopic cohort of small children although the pet ownership was relatively low. Further studies will focus on community distribution of pet allergens and possible cross-reactivity of pet and food allergens.

748

The pattern of respiratory allergy among underprivileged children from the city of Ica, Peru, South America

Holloway, EH¹; Yonz Pineda, PO²

¹Department of Paediatric Allergy, St. Thomas' Hospital, London, United Kingdom; ²Centro Nacional de Salud Intercultural (CENSI), Instituto Nacional de Salud, Lima, Peru

Background: The city of Ica, Peru, is a regional capital in the arid coastal zone of Peru 350 km south of the capital, Lima. Common poverty-related diseases such as malnutrition and tuberculosis are endemic. However, there is also a high incidence of chronic respiratory symptoms attributed to allergy. These children are invariably blindly diagnosed without allergy testing. We sought to carry out skin prick tests to identify children who have genuine respiratory allergy and would therefore benefit from allergen reduction measures with/without medication.

Table 1 Demonstrates the prevalence (% with 95% CI) of asthma, allergic rhinitis and eczema in 2003 and 2012.

	2012 (6–7 year)	2003 (6–7 year)	2012 (13–14 year)	2003 (13–14 year)
Diagnosed asthma	5.4 (4.7–6.1)	3.3 (2.7–3.9)	5.6 (4.7–6.5)	3.3 (2.7–3.9)
Current wheezing	10.7 (9.6–11.8)	6.9 (6.0–7.8)	12.0 (10.7–13.4)	5.1 (4.3–5.9)
more than 4 asthma attacks/past yr.	1.1(1.0–1.2)	0.9 (0.8–1.0)	1.3 (1.1–1.5)	0.7 (0.5–0.9)
Diagnosed hay fever	7.0 (6.1–7.9)	2.8 (2.3–3.3)	8.6 (7.5–9.7)	3.8 (3.2–4.4)
Current sneezing	16.4 (15.0–17.8)	8.7 (7.6–9.8)	28.4 (26.3–30.5)	15.3 (13.9–16.7)
Current rhinoconjunctivitis	5.2 (4.5–5.9)	2.9 (2.4–3.4)	9.6 (8.4–10.8)	4.5 (3.8–5.2)
Diagnosed eczema	4.4 (3.7–5.1)	1.8 (1.5–2.1)	2.3 (1.8–2.8)	1.6 (1.3–1.9)
Current itchy rash	4.4 (3.7–5.1)	3.4 (2.8–4.0)	7.2 (6.2–8.2)	2.5 (2.0–3.0)
Flexural dermatitis	3.6 (3.0–4.2)	2.4 (1.9–2.9)	5.0 (4.2–5.8)	1.8 (1.5–2.1)

Method: Children attending free medical clinics (run by MedICA, a local NGO) at two locations in Ica had a focussed allergy history taken and were offered the opportunity to have skin prick tests to 5 respiratory allergens: *D. pteronyssinus* (Dp), *D. farinae* (Df), *Blomia tropicalis* (Bt), *Periplaneta americana* (Pa) and *Blatella germanica* (Bg).

Results: 37 children underwent complete skin prick testing. 36 results were obtained of which 8 were positive. 5 children tested positive both to Df and Dp, 1 to Dp alone, 1 to Bt alone, 1 to Bg alone. No children had histories suggestive of pollen-related allergy. 1 child had a history also suggestive of dog allergy.

Of the 20 children who had been prescribed medication (anti-histamines/oral corticosteroids) only 6 (30%) tested positive. Of the 7 children who had been prescribed multiple medications, none tested positive. Nasal inflammation/obstruction was the most predictive clinical sign/symptom (4 of 7 were positive, 57%) whereas rhinorrhea, daytime or nighttime cough were poorly predictive of testing positive (23%, 22%, 7% respectively). There was a discrepancy in percentage of positive tests between the two clinic locations (29% for inner city district of Manzanilla, 15% for shanty town district of Parcona).

Conclusion: To the best of our knowledge, this is the first study of Paediatric respiratory allergy conducted in Ica, Peru. We have shown that many children being prescribed anti-histamines (mostly sedating type) and/or oral corticosteroids do not test positive to HDM or cockroach despite perennial symptoms. These children most likely have symptoms attributable to environmental irritants and pollutants than genuine allergic disease. We conclude that children should have allergy testing before being prescribed potentially harmful medication. Nasal obstruction is an important clinical sign that may predict respiratory allergy in this population.

749

Cord blood LC-PUFA composition and allergic diseases during the first 10 years of life. Results from the longitudinal LISAPLUS study

Standl, M¹; Demmelmaier, H²; Koletzko, B²; Heinrich, J³

¹IBE Chair of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; ²Dr. von Hauner Children's Hospital, University of Munich Medical Centre, Munich, Germany; ³Helmholtz Zentrum München – German Research Centre for Environmental Health, Institute of Epidemiology I, Neuherberg, Germany

Background: It has been suggested that n-6 and n-3 long-chain polyunsaturated fatty

acids (LC-PUFAs) in blood are associated with allergic diseases, although results are inconclusive. Low levels of n-6 LC-PUFA and high levels of n-3 LC-PUFA are anticipated to have beneficial effects. Pregnancy and lactation period may be critical. In this study, we examined whether n-6 and n-3 LC-PUFA levels in serum cord blood are associated with atopy and allergic diseases up to the age of 10 years.

Method: This analysis included 406 children from the Munich LISAPLUS birth cohort study. Information on doctor-diagnosed asthma, hay fever and eczema was collected using questionnaires completed at the ages 6 and 10 years, and for eczema additionally at 2 years. Specific IgE against inhalant allergens was measured at 6 and 10 years. Fatty acid composition was measured in cord blood and in blood collected at 2, 6 and 10 years. Associations between n-3 and n-6 LC-PUFAs in cord blood and allergic diseases or atopy were assessed using generalised estimating equations (GEE) considering the longitudinal structure. Models were adjusted for parental education, sex, BMI, time of follow-up (2, 6 or 10 years), age, maternal BMI before pregnancy, maternal atopy, and for PUFA composition at follow-up as sensitivity analysis.

Results: Cord blood n-6 LC-PUFA concentration had a significant protective effect on hay fever [adjusted OR (95%-CI): 0.73 (0.57–0.93), $P = 0.0125$], which did not change after adjusting for LC-PUFA composition at follow-up [0.73 (0.57–0.94), $P = 0.0141$]. The protective effect on allergic sensitisation against inhalant allergens were marginally significant [0.87 (0.75–1.02), $P = 0.0861$], also after adjustment for LC-PUFA composition at follow-up [0.86 (0.74–1.00), $P = 0.0507$]. Neither cord blood n-3 nor n-6 LC-PUFA was associated with eczema. There was no significant association between n-3 or n-6 LC-PUFAs in cord blood and asthma.

Conclusion: There is no indication of a beneficial effect of increased n-3 LC-PUFA in cord blood on the development of any of the disease entities.

750

Long-term effect of cow milk protein hydrolysates in high-risk children. Results from the 10-years follow-up of the GINI study

von Berg, A¹; Filipiak, B¹; Koletzko, S²; Krämer, U³; Hoffmann, B³; Link, E³; Beckmann, C¹; Hoffmann, U^{4,5}; Reinhardt, D²; Gröbl, A⁴; Heinrich, J⁵; Wichmann, E⁵; Bauer, CP⁴; Berdel, D¹

¹Marien-Hospital-Wesel, Pediatrics, Wesel, Germany; ²Ludwig-Maximilians-University, Dr. von Haunersches Kinderspital, Munich, Germany; ³IUF – Leibniz Research Institute for Environmental Medicine at the Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; ⁴Department of Pediatrics, Technical University of Munich, Munich, and LVA Oberbayern, Munich, Germany; ⁵Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Epidemiology I, Neuherberg, Germany

Background: The long-term impact of early intervention with hydrolysed infant formulas on allergic manifestations in high risk children is uncertain.

Objective: To investigate the effect of feeding children at risk for allergic diseases with different hydrolysed infant formulas in the first four months of life on allergic phenotypes until school-age.

Method: We used data of the ongoing German Infant Nutritional Intervention program (GINI) after 10 years of follow-up. Between 1995–1998, 2252 newborns with a familial risk for atopic diseases were recruited to participate in the prospective, randomised, double-blind intervention study GINI. At birth, children were randomly assigned to receive for the first 4 months one of four blinded formulas as breast milk substitute if necessary: partial (pHF-W) or extensive whey hydrolysate (eHF-W), extensive casein hydrolysate (eHF-C), or standard cow milk formula (CMF). Children were followed by modified ISAAC questionnaires and physical examinations at regular intervals. Outcomes were parent reported physician diagnosed atopic diseases. To examine the potential influence of the study formulas on the cumulative incidence from birth to 10 years in longitudinal analyses generalised estimation equation models were used. The prevalence at school-age (age 7–10 years) was estimated as period prevalence in the participants of the 10 year follow-up and analyzed by binomial regression models.

Results: In the intention-to-treat analysis ($n = 2252$) the relative risk (95% confidence interval) for the cumulative incidence of any atopic disease was 0.87 (0.77–0.99) for pHF-W, 0.94 (0.83–1.07) for eHF-W and 0.83 (0.72–0.95) for eHF-C compared with CMF. The corresponding figures for atopic eczema were 0.82 (0.68–1.00), 0.91 (0.76–1.10) and 0.72 (0.58–0.88), respectively. The effects were stronger in the per-protocol analysis ($n = 988$). The period

prevalence of atopic eczema at 7–10 years was significantly reduced with eHF-C in the per-protocol analysis. There was no preventive effect on asthma or allergic rhinitis.

Conclusion: The significant preventive effect on the cumulative incidence of atopic diseases, particularly eczema, with pHF-W and eHF-C persisted until 10 years without rebound, while eHF-W showed no signifi-

cant risk reduction. There is insufficient evidence for ongoing preventive activity at 7–10 years.

Poster Session 21

Infection and allergy

752

***In silico* investigation of oligonucleotide single stranded RNA immunostimulatory motifs as a major cause of CpG dinucleotide depletion in Human rhinovirus genomes**

Megremis, S¹; Demetriou, P¹; Manoussaki, A¹; Passioti, M¹; Trochoutsou, K¹; Taka, S¹; Papadopoulos, NG¹
¹Allergy Department 2nd Pediatric Clinic, University of Athens, Athens, Greece

Background: We have recently demonstrated that Human rhinovirus (HRV) genomes are characterised by a marked depletion of CpG dinucleotides. Basic viral evolutionary constraints such as codon usage or RNA secondary structures could not be incriminated in this process. The coding regions of many human innate immunity genes, particularly type I interferons, also have an extremely low CpG content, and mRNAs heavily expressed during the acute phase of the innate response have a bias towards low CpG content. We hypothesise that both HRV and host genes involved in the innate immune response have evolved to have low CpG content to avoid a CpG RNA sensing receptor.

Method: To test this hypothesis, 115 HRV fully sequenced genomes were investigated computationally for their capacity in hexamer-CpG/UpA-rich oligonucleotide motif sequences (6mer-ORN7) which have been implicated in the specific activation of Toll-like receptor 7 (TLR7) by ssRNA viruses.

Results: The genome distribution of 6mer-ORN7s follows the exact distribution of CpG dinucleotides in all HRV groups implicating sequence dependent recognition of HRV by the innate immune system as a major candidate for the observed CpG genome depletion and as a major viral evolution selective pressure. The analysis showed a differential motif-sequence organization between HRV-A and HRV-B genomes ($P < 0.05$). Inter-genome region-specific differences ($P < 0.05$) were observed in 5'UTR, 3A, 3C, 3D and 3'UTR among HRV-A and HRV-B, in VP4, 2B, 3A and 3B between HRV-A and HRV-C and in 5'UTR, 2B, 3A and 3D in HRV-B and HRV-C. The non structural 3D genome region was differentially rich in 6mer-ORN7s in all three HRV groups.

The most 6mer-ORN7-enriched genome region was the 5'UTR, while VP4 was almost totally depleted from these sequences in all three HRV clusters followed by extremely low motif numbers in the rest of the capsid encoding sequence. Sequence alignments indicated that HRV-A 6mer-ORN7s have the highest distribution in different genome locations. The highest 6-ORN7 motif homology was observed in 5'UTR in all three HRV groups (60–90%) with HRV-B genomes having a second distinctive motif with homology reaching 60%.

Conclusion: This study provides novel evidence that innate immune stimulation by viral ssRNA CpG ORNs is a strong HRV selective pressure.

753

Rhinovirus-induced type I interferon responses and viral load in primary nasal epithelial cells of subjects with or without atopic rhinitis and asthma

Spyridaki, IS¹; Skevaki, CL¹; Trochoutsou, AI¹; Megremis, S¹; Taka, S¹; Roumpedaki, E¹; Manoussaki, AE¹; Emmanouil, P²; Bakakos, P²; Loukides, S³; Papadopoulos, NG¹
¹2nd Department of Pediatrics, University of Athens, Athens, Greece; ²1st Respiratory Medicine Department, University of Athens, Athens, Greece; ³2nd Respiratory Medicine Department, University of Athens, Athens, Greece

Background: Defective type I interferon (IFN) production and consequent enhanced viral load have already been described in the bronchial epithelium of atopic asthmatic patients. The aim of the present study was to evaluate rhinovirus (RV) mediated IFN- β expression and RV load in upper airway epithelial cells of individuals with or without atopic rhinitis and asthma.

Method: Primary nasal epithelial cells were collected with the use of a curette from adults with atopic rhinitis ($n = 7$), atopic rhinitis and asthma ($n = 11$) and from non-atopic, healthy volunteers ($n = 8$). Donors were subjected to skin prick testing and a brief medical history was recorded for the assessment of allergic sensitisation and rhinitis. Lung function was evaluated by means of spirometry. Cells were exposed to 1 multiplicity of infection of RV1b or control medium. Culture supernatants and

total RNA were harvested after incubation for 6–72 h. RV-induced cytotoxicity was evaluated by a crystal violet colorimetric assay and RV-mediated RANTES release in cell supernatants was determined with the use of ELISA. In order to investigate viral load we evaluated the intracellular RV RNA levels by real-time PCR. RV-induced IFN- β expression was also measured by real-time PCR.

Results: RV infected cells did not exhibit significant differences in terms of cytotoxicity between the three groups at any time-point examined. RV-mediated RANTES production by rhinitic donors was higher than control cells at 72 h post-infection ($P < 0.001$) and there was a trend for accentuated release by asthmatic donors that did not reach statistical significance. Infected cells from asthmatic donors displayed enhanced RV RNA levels at 24 h compared to the other two groups ($P < 0.05$), while at 48 h we found no significant differences. IFN- β mRNA expression at 6 h and 8 h after RV infection was significantly higher among rhinitic as compared to healthy individuals ($P < 0.05$). Cells from asthmatics expressed significantly lower IFN- β ($P < 0.05$) at 6 h, while at 8 h they reached similar to the control group expression levels.

Conclusion: Impaired IFN- β antiviral response of asthmatic nasal epithelium may account for the presence of higher RV load in this clinical group. Additional data are required to explain the enhanced IFN- β expression by RV-infected cells of patients with allergic rhinitis, resulting to viral loads comparable to the control group.

754

IL-10 interaction with innate IFNs in rhinovirus-infected monocyte-derived macrophages

Bocsan, IC¹; Makrinioti, H²; Nikonova, A²; Buzoianu, AD¹; Stanciu, L²; Johnston, S²
¹Clinical Pharmacology, University of Medicine and Pharmacy, Cluj Napoca, Romania; ²NHLI, Imperial College, London, United Kingdom

Background: Rhinoviruses (RVs) consist the major cause of asthma exacerbations in both children and adults. Our group and others have shown that asthmatics have

deficient innate and adaptive immune response to rhinovirus. IL-10 is involved in down-regulation of mediators associated with Th1 responses and antiviral activity. It has been reported that the IL-10 gene expression has been up-regulated in monocytes/macrophages from patients with virus-induced asthma exacerbations, suggesting that IL-10 may play a significant role in rhinovirus-induced asthma exacerbations.

Aim: The aim of the study was to determine in vitro whether IL-10 treatment of RV-infected macrophages decreases innate IFNs.

Method: Monocytes were prepared from PBMCs and differentiated to macrophages by culturing with GM-CSF for 7 days. MDMs were exposed to RV16 1 MOI for 1 h and after that were treated with IL-10 in different concentrations (1, 10 and 100 ng). Cell lysates and supernatants were collected at various time points after the infection (0, 2, 8, 24 and 48 hours). RNA was extracted from cells and used to assess viral RNA and IL-10, IFN- $\alpha/\beta/\lambda$ mRNA expression by qPCR Taqman. Supernatants will be used to titrate RV16 release and to measure cytokines production by ELISA.

Results: RV16 increases the interferon's level in infected MDM at 8 and 24 hours after infection. IL-10 10 and 100 ng significantly decrease RV-induced IFN- α and beta mRNA expression at 8 hours and for IFN α at 24 hours also. Both concentrations significantly decrease also the expression of mRNA for IL-28 and IL-29, especially at 8 hours. The higher concentration of IL-10 decreases also the gene expression for IFN λ at 24 hours. The IFN α and beta levels are reduced significantly by the IL-10 100 ng at 24 and 48 hours, while the level of IL-29 is decreased only at 48 hours. The virus titer is increased at 8 hours in IL-10 100 ng treated MDM.

Conclusion: IL-10 increases virus titer and subsequently decreases RV-induced innate IFNs in MDMs.

755

Interferon-gamma-producing gamma delta T-lymphocytes, stimulated *ex vivo* with *M.tuberculosis* antigens, in whole blood of adolescents with active and latent tuberculosis infection

Mordovskaya, LI¹; Vladimirov, MA²; Aksanova, VA²
¹SBI 'Research-Practice Center 'Phthisiatry' of the Sakha Republic Yakutia, Yakutsk, Russia; ²Research Institute of Phthisiopulmonology, I.M. Sechenov First Moscow State Medical University, Moscow, Russia

Background: Exploration of the role of gammadelta T-cells during infectious pro-

cess is gaining more and more importance. In tuberculosis (TB) infection gammadelta T-cells are found circulating in blood and infiltrating the lung tissue, and represent one of the components (possibly the most important component at early infection stage) of both the innate and acquired cell-mediated antimycobacterial resistance. The induction, and subsequent proliferation and expansion, of these T-cells is induced by a wide spectrum of protein and non-protein (pyrophosphoric monoesters) mycobacterial antigens directly, bypassing MHC molecules.

Method: Immunophenotyping and analysis of the percentage of CD3 + gammadelta+IFN-gamma+ cells in 21 adolescent with active pulmonary TB and in 12 adolescents with latent TB infection prior to antibacterial therapy have been performed, to assess the intracellular production of IFN-gamma by PPD- and ESAT-6-stimulated gammadelta T-cells during short-time (6 hour) incubation in peripheral whole blood samples. Composition of peripheral blood lymphocytes was analyzed by direct immuno-fluorescence test, using monoclonal anti-bodies.

Results: Percentage of CD3 + gammadelta+ cells was significantly lower in blood of patients with pulmonary disease, than those with latent infection: 2.53 ± 0.3 and 4.93 ± 0.8 ($P < 0.05$), respectively. But when antigen-induced (PPD and ESAT-6) production of IFN-gamma was analyzed, the percentage of CD3 + gammadelta+IFN-gamma+ cells was significantly higher in pulmonary disease, compared to latent infection, both in control blood samples without antigen ($4.2 \pm 0.8\%$ vs. $1.55 \pm 0.3\%$, $P < 0.05$), and in samples induced with PPD and ESAT-6 ($9.3 \pm 1.0\%$ and $8.7 \pm 0.7\%$ vs. $3.8 \pm 1.2\%$ and $4.5 \pm 1.5\%$, $P < 0.05$, respectfully).

Conclusion: We found increased number of IFN-gamma producing T-cells during active TB, compared to that in latent TB infection. Gammadelta T-cell subset count decreases during active TB, but within this subset, the proportion of IFN-gamma producing cells is considerably higher in active TB, and the proportion of IFN-gamma producing cells in samples incubated in the presence of *M.tuberculosis* antigens was likewise increased. Immunophenotyping of T-cells, and gammadelta T-cell subset in particular, opens additional opportunities for assessment of specific immune response and for differentiation between active and latent tuberculosis infection.

756

Triggering receptor expressed on myeloid cells -1 (TREM-1) expression on neutrophils and monocytes in pregnancy at risk of preterm delivery

Ierullo, AM¹; De Amici, M²; Perotti, F¹; Torre, C²; Spiniello, A¹

¹Fetal Medicine Unit, Fondazione I.R.C.C.S Policlinico San Matteo, Department of Obstetrics & Gynecology, Pavia, Italy; ²Fondazione I.R.C.C.S. Policlinico San Matteo, Department of Paediatrics and Neonatology, Pavia, Italy

Background: Triggering receptor expressed on myeloid cells -1 (TREM-1) is a pattern-recognition receptor expressed on neutrophils and monocytes. It participates in innate immune responses and it is activated in the event of disseminated bacterial infections being and its expression is up-regulated leading to production of proinflammatory mediators. We aimed to test the hypothesis that TREM-1 expression may be elevated during pregnancy as manifestation of physiologic activation of the immune response and to compare those values in those pregnancies at higher risk of pre-term delivery before 34 weeks of gestational age.

Method: Venous serum samples were collected from 17 pregnancies at risk of pre-term delivery (PTD) (Group C) diagnosed before 34 weeks' gestational age (GA), 26 normal GA-matched pregnancies (Group B), and 41 fertile non pregnant women (Group A). TREM-1 on neutrophils was measured with a Quantikine[®] ELISA (R&D Systems[®], Minneapolis, MN, USA).

Results: The TREM-1 values (pg/ml) [mean;SD] showed no differences among the groups [(A = 3196;103); (B = 3093;716); (C = 2479;613);]. Serum IL-8 [mean;SD] appears to be elevated in the uncomplicated pregnancies than in PTD [(65.3; 81.9) vs. (34.2;60.5) n.s.]. Cervical length is shorter in the PTD than in complicated pregnancies [(18; 8.8) vs. (39.6;7.4) n.s.].

Conclusion: TREM-1 values expressed on neutrophils appeared to be similar in non pregnant women, in singleton uncomplicated pregnancies and in those at high risk of pre term delivery. On the contrary IL-8 production appeared to be elevated in uncomplicated singleton pregnancies.

757

Effects of penicilin and montelukast Na on middle ear mucosa in rats with experimental acute otitis media

Seçil Uçar Topalsan, S¹; Yalcin, AD²; Tural Huseynov, T¹; Sarıoğlu, S³; Serbetcioglu, B¹
¹Dokuz Eylul University, Medical School, Department of Otorhinolaryngology, Izmir, Turkey; ²Clinical Immunology and Allergy Unit of Department of Internal Medicine, Antalya Training and Research Hospital, Antalya, Turkey; ³Dokuz Eylul University, Medical School, Department of Pathology, Izmir, Turkey

Background: To develop an experimental acute suppurative otitis media model and compare the responses of rats to penicillin and combinations of leukotriene antagonist with respect to histopathological observations conducted at both early and late phases.

Method: Fifty six female Wistar rats (weight 200–250 g) were used for this study. They were kept under standard laboratory conditions and given food (pellets) and water ad libitum. All ears of the rats examined by otomicroscopy and 83 ears that are free of middle ear infections were included. All animals were anesthetized with a combination of 50 mg/kg ketamine hydrochloride (Ketalar[®]) and xylazine hydrochloride given intraperitoneally. All prosedure were performed under sterile conditions. Pneumococcal otitis media was induced in each ear by transtympanic inoculation with 0.03 ml of suspension of type 3 Pneumococci (ATCC 49619), at a concentration of 10⁹ CFU /ml). The animals were initially examined at 48 hours post-inoculation by otomicroscopy and confirmed the presence of otitis media. The animals were randomly divided into four groups: Group A (*Antibiotic-treated group*): Two days after inoculation, 14 animals (20 ears), were treated with intramuscular penicilin G 160.000 U/kg once daily for five days. Group B (*Antibiotic and montelukast co-treated group*): Two days after inoculation 14 animals (23 ears), were treated with Pen G and Montelukast Na (10 mg/kg/day, 21 days, (Y)) intraperitoneally. Group C (*montelukast treated group*): Two days after inoculation 14 animals (21 ears), were treated with Montelukast Na (10 mg/kg/day, 21 days) and Group D (*placebo group*): Two days after inoculation, 14 rats (20 ears), were not given any medication, only 2 cc phosphate saline intraperitoneally. Two rats, one in antibiotics and montelukast co-treatment group, one in infected controls were died, and excluded from the study.

Results and conclusion: No significant difference was found between the groups, apart from mucosal vascularization with respect to mucosal and TM parameters at early phases. However, statistically

significant differences were found for the improvement of TM thickness with the help of penicillin treatment. Furthermore, considerable deviations were observed for the recuperation of TM and mucosal inflammation for groups where subjects were injected with montekulast as compared to other groups of the study.

758

In vitro antifungal effect of cyclosporine A against *Malassezia pachydermatis* isolated from the skin of dogs with atopic dermatitis

Brazis, P¹; Ramió-Lluch, L¹; Cerrato, S¹; Puigdemont, A²
¹Univet, I+D, Barcelona, Spain; ²Universitat Autònoma de Barcelona, Department of Pharmacology, Toxicology and Therapeutics, Barcelona, Spain

Background: *Malassezia* yeast is usually part of the dogs' skin resident microflora. Under certain conditions, *Malassezia pachydermatis*, the most commonly isolated specie in dogs, can become pathogen exacerbating atopic dermatitis (AD) signs. In dogs, ketoconazol is considered the treatment of choice against *Malassezia*. Previous studies have demonstrated that the calcineurin inhibitor tacrolimus inhibits *Malassezia* growth. The mechanism of action seems to be related to the presence of a calcineurin homologue in the fungal cells. Cycloporine A (CsA), another calcineurin inhibitor, has been recently formulated using nanotechnology techniques to allow their topical treatment of AD lesions in dogs.

The aim of this study was to evaluate the *in vitro* antimicrobial efficacy and the minimal inhibitory concentration (MIC) of this new topical formulation of CsA, in comparison to ketoconazol and tacrolimus. **Method:** Ten *Malassezia* isolates were obtained from the skin of dogs with AD from the Clinical Hospital of the Veterinary School of Barcelona. Ketoconazol, tacrolimus and CsA were diluted in Sabouraud broth to obtain final concentrations ranging from 0.016 to 128 µg/ml. *Malassezia* isolates were inoculated to Sabouraud-treated and control (drug-free) broth and incubated in the dark at 35°C for 7 days. Yeast growth in treatment and control samples was recorded every 24 h according to a scale from 0 (no growth) to 4 (growth similar to control).

Results: All *Malassezia* strains were sensitive to the 3 tested drugs. Ketoconazole showed MICs values (MIC range from 0.016 to 0.032 µg/ml) significantly lower than calcineurin inhibitors. New CsA formulation and tacrolimus also showed a significant antifungal effect against *Malassezia* strains, with MIC values rang-

ing from 32 to 128 µg/ml. Moreover, 70% of the *Malassezia* strains were inhibited at a MIC of 64 µg/ml by both drugs.

Conclusion: This is the first study evidencing the potential antifungal effect of CsA, against *Malassezia pachydermatis*. CsA has been used in human and veterinary medicine to control AD. Given that *Malassezia* usually exacerbates AD, the growth-inhibitory effect of CsA against this yeast, reinforces its usefulness as a topical treatment in atopic dogs skin.

761

Analysis of the clinical features and treatment response of toxocariasis in adults

Kim, J-H¹; Park, S¹; Hwang, YI¹; Jang, SH¹; Jung, K-S¹; Kim, D-G¹
¹Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Anyang, Korea

Background: Toxocariasis is a zoonotic infection caused by *Toxocara canis* (*T. canis*) and *Toxocara cati*, which can cause blood eosinophilia and eosinophilic infiltration in internal organs. This study was to investigate clinical features and treatment response of toxocara infection in adult patients.

Method: We enrolled the subjects diagnosed as having toxocara infection from 2007 to 2012, one tertiary hospital in Korea. A diagnosis of toxocariasis was made by enzyme-linked immunosorbent assay (ELISA) using that secretory-excretory antigen from the *T. canis* larvae. Laboratory data were collected including peripheral eosinophil count, total IgE, and ELISA tests for parasites (*Cysticercus*, *Paragonimus westermani* and *Clonorchis sinensis*). Computed Tomography (CT) scan of chest or liver was performed to find tissue infiltration at diagnosis. Clinical, laboratory and radiologic data were followed up after treatment with an interval of 3 months.

Results: A total of seventy three patients were enrolled in this study. Fifty eight patients had lung involvement (79.5%, group I), eight with liver involvement (11.0%, group II) and seven with asymptomatic blood eosinophilia (9.6%, group III). 30.1% of all subjects were asymptomatic. Patients in group I showed clinical symptoms such as hemoptysis, chest pain, and cough compared to those of group II and III (*P* = 0.018). The median values of eosinophil count and total IgE level in group II were highest among three groups at diagnosis (*P* < 0.001 for eosinophil count and *P* = 0.018 for total IgE, respectively). All patients showed decreased

eosinophil counts with symptom improvement after treatment with a 7-day course of albendazole (400 mg twice daily, $P < 0.0001$). During the follow-up period, forty three patients were taken serial CT scan, showing 81.4% of them disappearance of tissue infiltration in their lung or liver. However, eight patients (13.7%) in group I and one (12.5%) in group II failed to complete radiological regression, requiring an additional course of albendazole with systemic corticosteroid.

Conclusion: The most common clinical manifestation of toxocariasis in adults was a lung infiltration with mild respiratory symptoms, whereas systemic eosinophilic inflammation was more prominent in patients with liver infiltration. A 7-day course of albendazole was effective in most cases; however, 10% of the patients needed systemic corticosteroid for both clinical and radiological resolution.

762

Combination of a corticosteroid and an anti-viral intranasal therapy

Nakowitsch, S¹; Koller, C¹; Kurz, M¹; Fazekas, T²; Mueller, CA³; Prieschl-Grassauer, E¹

¹Marinomed Biotechnology, Vienna, Austria; ²St. Anna Children's Hospital, Vienna, Austria; ³Department of Otorhinolaryngology, Medical University of Vienna, Vienna, Austria

Background: Intranasal corticosteroids (INCS) provide potent, localised treatment for allergic rhinitis, idiopathic rhinitis, rhinosinusitis and nasal polyposis with minimal risk of systemic exposure. However, corticosteroids cause immunosuppression and can decrease the anatomical barrier function of the skin. Consequently, infection and multiplication of all types of pathogens (bacteria, viruses and fungi) may occur, while alleviating inflammation. Here we introduce the concept of creating a protective physical barrier in the nasal cavity with an antiviral polymer (carrageenan) that works as inhibitor against respiratory virus entry for prophylaxis and therapy. Independent clinical trials in adults and children revealed that the intranasal administration of iota-carrageenan significantly reduced the time to disease clearance of patients with common cold and decreased the virus load in nasal lavages. Iota-carrageenan is already marketed in Europe as antiviral active component of medical device nasal sprays under different brand names.

Method: We suggest the combination of budesonide, a well described corticosteroid with a long history of use as intranasal drug, with iota-carrageenan in a nasal spray. A combined formulation of budesonide and iota-carrageenan was *in vitro*-

tested for its antiinflammatory activity by inhibiting TNF-alpha release of LPS induced whole blood. Accessorily, the combination was tested for its anti-viral effectiveness against human rhinovirus (HRV1a and HRV8) on HeLa cells and influenza virus on MDCK cells.

Results: Both substances – budesonide and iota-carrageenan – showed irrespectively of their combination an unmodified mode of action *in vitro*.

Conclusion: Thus, patients would benefit from a fast treatment of symptoms of rhinitis together with an efficient anti-viral prophylaxis and treatment.

763

Azithromycin is able to augment interferon and interferon stimulated gene responses to rhinovirus *in vitro*

Porter, JD¹; Macintyre, J¹; Sykes, A¹; Gupta, A¹; Shoemark, A¹; Bossley, C¹; Davies, J¹; Khaitov, M²; Moshkovskii, S³; Kon, OM⁴; Saglani, S¹; Bush, A¹; Johnston, SL¹; Edwards, MR¹

¹Imperial College London, NHLI, London, United Kingdom; ²NRC Institute of Immunology FMBA, Moscow, Russia; ³Department of Proteomics, Institute of Biomedical Chemistry Moscow, Moscow, Russia; ⁴Imperial College London, NHS Trust, London, United Kingdom

Background: Asthma exacerbations (AE) carry a high rate of patient hospitalisation and also a high economic burden. Current therapies for AE are only partially effective in controlling mortality and morbidity associated with the disease and thus novel therapies are sought-after. Rhinovirus (RV) infection strongly associates with AE and asthmatic-derived cells have been shown to be deficient in interferon (IFN) production in response to RV *in vitro*, thus IFNs may provide a novel therapeutic for RV-associated AE. Macrolides have antiviral and anti-inflammatory effects. Azithromycin is able to augment the expression of RV-induced type I and type III IFN (IFN β and IL-28/IL-29) and several interferon stimulated genes (ISGs) in primary human bronchial epithelial cells (HBECS). In this study we attempt to understand the mechanism of Azithromycin and assess its effects on atopic asthmatic cells, we hope to determine whether azithromycin would be suitable as a novel therapeutic course in the treatment of RV-induced AE.

Method: Normal HBECS or cells from bronchial brushings of mild/moderate asthmatic adults or severe asthmatic children were cultured with Azithromycin for 24 h prior to infection with RV1b. qPCR and western blots were used to determine gene/protein expression. Virus replication was measured by TCID₅₀ of HeLa cells cultured with titrated HBEC supernatants.

Results: Azithromycin augmented RV16 and RV1b-induced expression of IFNs; IFN β (1.40 and 1.75 fold), IL-28 (1.10 and 2.05 fold), IL29 (1.19 and 2.68 fold) and anti-viral ISGs; Viperin (4.40 and 2.11 fold), MxA (3.12* and 4.26* fold) (* $P < 0.05$ $n = 7$) in atopic asthmatic HBECS. Azithromycin can reduce viral release in these cells by 42%* after 48 h (* $P \leq 0.05$, $n = 6$). Azithromycin had no effect on the expression IFNs or ISGs when used alone and only augmented RV-induced IFN expression. Azithromycin augmented IFN responses in normal HBECS via the RIG-like helicase pathway, possibly by preventing degradation of the RIG-I/MDA-5 adapter protein Mitochondrial-associated Anti-Viral Signaling protein (MAVS).

Conclusion: Azithromycin is able to augment IFN responses in primary HBECS from asthmatics when infected with RV, the observed decrease in viral replication with azithromycin highlights a novel virus-dependent therapeutic avenue for the treatment of RV-associated AE. The mechanism of action is still not fully understood however our data suggests that the IFN augmenting effects of Azithromycin are via the RIG-like helicase pathway in HBECS.

764

The level of Ig E in patients with cystic fibrosis associated with Pseudomonas aeruginosa pulmonary infection

Sciuca, S¹; Dimitrova, O¹; Cotoman, A¹; Eremciuc, R¹; Lupusor, N¹; Macovei, T¹

¹State University of Medicine and Pharmacy 'Nicolae Testemitanu', Pediatric, Chisinau, Moldova

Background: About 60% of people with cystic fibrosis have a chronic respiratory infection caused by a bacteria called Pseudomonas aeruginosa. Once it sets up house in the respiratory tract, Pseudomonas aeruginosa is hard to get rid of and on long time persistence it can cause immunopathological disorders.

Aim: The study of reagenic immunopathological mechanisms in patients with cystic fibrosis (CF) with Ps. aeruginosa pulmonary infection.

Method: The research included 53 patients with cystic fibrosis with age variety from 1 till 25 years, most of them (22) younger than 7 years old. The group of study was divided in two lot of studies, the first one – 40 children with Ps.aeruginosa, and the second – 13 children without Ps.aeruginosa. Clinical associations between cystic fibrosis and bronchial asthma were represented in six children, allergic rhinitis in 10 children in the first group of study by comparison with any case of the second group.

The severity of immunopathological disorders is confirmed by the high level of IgE. In more of 1/2 cases the growth and the development disturbances were presented. The determination of summary IgE in serum was carried out by immunoenzyme method with the utilisation of standard tests.

Results: The concentration of IgE in serum for all children from the lot of study with pulmonary infection with *Ps.aeruginosa* was considerable increased – 252.76 ± 11.47 UI/ml ($P < 0.001$) in com-

parison with the lot without *Ps.aeruginosa* (65.9 ± 5.77 UI/ml).

Immunological researches in the lot of study with *Ps.aeruginosa* had determinated hyperimmunoglobulinemia E in 54.8% children with 46.4–1358.6 UI/ml varieties. Also, the level of hyperimmunoglobulinemia E grows with the age of children: in 45.71% – younger then 7 years was determinated the average level of IgE – 44.6 UI/ml; in 28.57% pupils with the age between 7–14 years with the average level of IgE – 290.6 UI/ml and in 25.71% – adolescents older then 14 years

with the average level of IgE – 548.13 UI/ml.

Conclusion: We had observed that the level of IgE depends of the long time persistence of the infection with *Ps.aeruginosa*. The immunopathological mechanism of the hyperimmunoglobulinemia E in children with cystic fibrosis is determinated by the presence and the long time persistence of the lung infection with *Ps.aeruginosa* which one explain the increased incidence of bronchial asthma.

Poster Session 22

Tools for improving laboratory and clinical allergy diagnosis

765

Distinguish cross reactivity from true co-sensitisation is crucial for the immunotherapy of weed pollenosis patient

Park, H-J; Park, KH; Lee, J-H; Hong, C-S; Park, J-W
Division of Allergy and Clinical Immunology,
Department of Internal Medicine, Yonsei University
College of Medicine, Seoul, Korea

Background: Ragweed and mugwort are the major allergenic weed pollens in Korea. Allergen specific immunotherapy (IT) is available for these two pollen allergies. To select culprit allergens for IT, detection of sIgEs against responsible allergens are crucial. In this study, we evaluated clinical relevance of measurement of specific IgE to group 1 major allergens of these pollens.

Method: We enrolled 107 patients who have allergic symptoms in last summer and divide them into three groups according to their skin prick test (SPT) results; ragweed mono-sensitisers (RMS), mugwort mono-sensitiser (MMS) and co-sensitised patient to both allergens (CS). We measured sIgEs to whole extract of ragweed and mugwort pollens, and group 1 major allergens (Amb a 1 and Art v 1, respectively). And to distinguish cross reactivity from true sensitisation, we performed CAP inhibition assay.

Results: In RMS group ($n = 27$), detection rates of sIgE of whole ragweed and Amb a 1 allergen were 63.0% and 55.5%, and two patients were Amb a 1 sIgE positive with negative response to ragweed. In MMS group detection rate of sIgE to whole mugwort and Art v 1 were 67.6% and 54.1%, and all Art v 1 positive patients were also positive to mugwort sIgE. In CS group, concordance rate between sIgE of ragweed and Amb a 1 is 53.5%, mugwort and Art v 1 is 74.4%. In the discordance sera with positive to ragweed but negative to Amb a 1 sIgE ($n = 7$), CAP inhibition test using mugwort allergen as a inhibitor, ragweed sIgE were inhibited more than 50% in six patients (85.7%). In and In discordance serum with positive sIgE to mugwort but without Art v 1 sIgE ($n = 2$), ragweed allergen inhibited mugwort sIgE more than 60% in all two patients.

Conclusion: SPT may insufficient to detect genuine culprit allergens of weed pollenosis patients. Measurement of sIgE of crude

extract and single major allergen is supplemental for diagnosis of weed pollen allergen, and presence of group 1 major allergen of ragweed and mugwort may imply true sensitisation and helpful for distinguish it from cross-reactivity in Korea.

766

Allergenic characterisation of a novel allergen, homologous to chymotrypsin, from German cockroach

Jeong, KY¹; Son, M¹; Kim, BJ²; Lim, K-J²; Lee, J-H¹; Hong, C-S¹; Park, J-W¹

¹Internal Medicine, Yonsei University College of Medicine, Seoul, Korea; ²ProteomeTech Inc., Seoul, Korea

Background: IgE reactive components are known to be rich in cockroach feces. Various protease allergens were identified by the proteomic analysis of German cockroach fecal extract in a previous study. In this study, we characterised a novel allergen, a chymotrypsin-like serine protease.

Method: A cDNA sequence homologous to chymotrypsin was obtained by the analysis of German cockroach expressed sequence tag (EST) clones. The recombinant protein was expressed in *Escherichia coli* using pEXP5NT/TOPO vector system, and its allergenicity was investigated by ELISA.

Results: Deduced amino acid sequence showed 32.7–43.1% identity with mite group 3 (trypsin) and group 6 (chymotrypsin) allergens. Sera from 8 of 28 subjects (28.6%) showed IgE binding to the recombinant protein. IgE binding to the recombinant cockroach chymotrypsin was inhibited by house dust mite chymotrypsin Der f 6, while it showed minimal inhibition to German cockroach whole body extract.

Conclusion: A novel allergen homologous to chymotrypsin and cross-reactive with Der f 6 was identified from German cockroach.

767

Evaluation of a non-commercial rapid point of care test for mast cell markers using tryptase as a model protein

Rydell, N¹; Broberg, J¹; Strandberg, N¹; Thorell, L¹; Sjölander, A¹

¹ThermoFisher Scientific, Uppsala, Sweden

Background: A rapid point of care test was investigated for its suitability to detect and quantify mast cell proteins in serum or plasma. Tryptase was used as a model protein. Tryptase is the most abundant protein in mast cells. Immature tryptase constitutively leaks into the plasma. Constantly elevated levels of tryptase may reflect an increased burden of mast cells and are associated with blood disorders such as mastocytosis. Upon mast cell activation (e.g. anaphylaxis), stored mature tryptase is released resulting in transiently elevated levels of tryptase with peak values typically between 15 and 120 min after the mast cell activation. The use of rapid point of care test with tryptase as a model protein was investigated and compared to a commercially available laboratory immunoassay for total tryptase.

Method: A capillary flow membrane assay was established. An antibody specific for all forms of tryptase was immobilised as thin bands on nitrocellulose membrane strips (one band per strip). A different antibody, also specific for all immature and mature forms of tryptase, was coupled to gold particles and used as detection antibody.

Serum samples and tryptase calibrators, 20 µL respectively, were applied to the strips and allowed to migrate by capillary flow. The gold conjugate and wash liquids were subsequently added to reveal red colored bands on the strips where the conjugate had bound. Evaluation of the assay was performed either visually or with a photometric color reader.

Using the color reader, a calibration curve was established that could be used to determine the tryptase concentration in serum. The results were compared with a commercial tryptase assay.

Results and Conclusion: Proof of principle was shown for a new rapid point of care assay for the measurement of mast cell proteases in serum or plasma using tryptase as a model protein. Assay results

could be obtained within 30 min using only 20 µL serum or tryptase calibrators and showed excellent conformity to the commercial tryptase assay.

768

An inhibition ELISA for the determination of major allergen group 1 in different grass species

Kerkvliet, E¹; Sinnige, N¹; Warmenhoven, H¹; Akkerdaas, J²; van Ree, R²; van den Hout, R¹

¹HAL Allergy BV, Development, Leiden, The Netherlands; ²Academic Medical Center, Amsterdam, The Netherlands

Background: Allergy induced by grass pollen can be reduced by means of sublingual allergen-specific immunotherapy (IT). One of the major allergens in grass pollen is grass Group 1. Two antibody-based methods, a sandwich-ELISA and an inhibition ELISA, were developed for the quantification of grass Group 1 in grass pollen extracts. Both methods were compared in order to determine the most appropriate assay for use of quantification of Group 1 in a grass pollen IT product that consists of four different grass pollen extracts.

Method: The sandwich ELISA uses two specific monoclonal antibodies (7E7 and 1B8) against Lol p1 major allergen, one on the solid phase and one as detecting antibody. The inhibition ELISA (iELISA) uses one specific monoclonal antibody (7E7) that is mixed with grass pollen extract and recombinant Phleum p1 allergen on the solid phase. The products tested were pollen extracts of four different grass species, i.e. *Phleum pratense*, *Lolium perenne*, *Poa pratensis* and *Secale cereale*. The standards used are well characterised grass Group 1 allergens (Phl p1, Lol p1, Poa p1 and Sec c1) purified from grass pollen extracts.

Results: The sandwich ELISA showed different curves (e.g. non-parallel lines, different OD maxima) when grass pollen extracts from different grass species were analysed, while the iELISA showed similar curves for all four grass species. Further development of the sandwich-ELISA would involve separate development of 4 methods with different conditions, while only one method could be used with the iELISA for these extracts of 4 grass species. Therefore, the iELISA was chosen for further development and qualification. Qualification showed that the iELISA generates reproducible results with low variation.

Conclusion: Quantification of grass Group 1 major allergen in four different grass species can be done with one method using an iELISA. The sandwich ELISA was less suitable since different test conditions were

needed when testing different grass species. The newly developed iELISA can precisely quantify Group 1 allergen in pollen extracts from *Phleum pratense*, *Lolium perenne*, *Poa pratensis* and *Secale cereale*.

769

Sensitisation to Act d 2 in patients allergic to *Alternaria alternata*: an epiphenomenon without clinical significance?

Sánchez-López, J¹; Pascual, M²; Gómez-Casado, C³; Muñoz-Cano, R¹; Rueda, M¹; Vilella, R²; Valero, A¹; Díaz-Perales, A³; Bartra, J¹

¹Pulmonology and Respiratory Allergy, Hospital Clinic, Barcelona, Spain; ²Hospital Clinic, Immunology, Barcelona, Spain; ³Centro de Biotecnología y Genómica de Plantas UPM-INIA, Campus de Montegancedo, Pozuelo de Alarcón, Spain

Background: In the last few years, the introduction of microarrays in the diagnosis of type I allergy is allowing the clinicians to have a much more accurate picture of their allergenic profile. However, the simultaneous measurement of specific IgE to multiple molecules can show unexpected sensitisations, without knowing their clinical relevance. For instance, we have been observing a high prevalence (74%) of sensitisation to Act d 2 (the thaumatin of kiwifruit) in patients sensitised to Alt a 1 (major allergen of *Alternaria alternata*) with a confirmed allergy to this mould. The aim of the present study was to clarify if there was any clinical relevance in this finding.

Method: We selected patients allergic to *A. alternata* (rhinitis and/or asthma) with a positive specific IgE (sIgE) to Alt a 1 and Act d 2 in the allergen microarray immunoassay (ImmunoCAP ISAC, ThermoFisher Scientific).

Skin prick tests (SPT) were performed with the commercial available *A. alternata* and kiwifruit extracts (Laboratorios LETI), a purified nAct d 2 protein and a prick by prick with fresh kiwifruit. Total IgE and sIgE (UniCAP, ThermoFisher Scientific) to kiwifruit and *A. alternata* were obtained, and an open oral food challenge (OFC) with kiwifruit was also carried out.

Results: Eighteen patients were selected, nine of them were skin prick tested and orally challenged. 9 men, 9 women, median total IgE 195 kU/l (interquartile range (IQR) 128–542). sIgE to kiwi was negative in all cases, median sIgE to *A. alternata* was 9.86 kU/l (IQR 5.5–16.8), median sIgE to Act d 2 was 1.9 ISU (0.9–4.6), and sIgE to Alt a 1 was 9.8 ISU (2.5–20.4). A correlation was found between sIgE to Act d 2 and sIgE to Alt a 1 (Rho Spearman = 0.686; significance = 0.01).

SPT to *A. alternata* extract was positive in all cases, SPT to commercial kiwi, prick by prick with fresh fruit and nAct d 2 were all negative. OFC with kiwifruit was well tolerated in all patients.

Conclusion: In our population, sensitisation to Act d 2 in the context of an allergy to *A. alternata* has no clinical relevance. This sensitisation could only be seen with the microarray immunoassay, SPTs and sIgE to kiwi failed to detect it.

A significant correlation was found between sIgE to Act d 2 and Alt a 1. Further studies are needed to confirm if this sensitisation is real and in second place, to figure out if there could be any other link between both allergens rather than the clinical one.

770

Stability of purified nsLTP prick tests by *in vitro* analysis

Moya, R¹; López-Matas, MA¹; Carnés, J¹

¹Laboratorios LETI, R & D, Tres Cantos, Spain

Background: The use of purified allergens as a diagnostic for skin prick test allows the identification of real sensitisation to a specific allergen present in a protein extract and it is a very useful tool for the study of cross-reactivity. However, the quality of these products has to be demonstrated. In that sense, there is no studies about the stability of purified proteins as reagents for diagnosis. The objective of the study was to determine the stability of purified nsLTP prick tests under different storage conditions.

Method: Pru p 3 and Cor a 8 were purified in an ÄKTAexplorer FPLC and used to prepare prick tests with a concentration of 30 µg/ml of total LTP. A mixture of purified LTPs (15 µg/ml of Pru p 3 and 15 µg/ml of Cor a 8) was selected in order to increase the diagnosis efficacy. After formulation, pricks were stored at three temperatures (room temperature, 4° and –20°C) and they were analysed monthly by SDS-PAGE and immunoblot. Pru p 3 or Cor a 8 polyclonal rabbit antibodies were used in immunoblot experiments. Finally, pH and glycerol valuation was also carried out.

Results: SDS-PAGE and immunoblot results showed that there were not important differences in protein molecular weights or protein band intensity between the 3 temperatures and during the whole period of study (6 months). Immunoblot results showed a slight decrease in the intensity of Cor a 8 recognition after 6 months. Regarding pH and glycerol values, both parameters were maintained in the range of 7.5–8.5 and 45–55%, respec-

tively, during the whole period and in the three temperature conditions.

Conclusion: Skin prick tests composed by purified Pru p 3 and Cor a 8 were stable after 6 months of being stored at room temperature, 4° and –20°C in terms of protein integrity, pH and glycerol content. Stability of purified LTPs as reagents for diagnosis is recommended in 6 months.

771

Recombinant production and immunologic characterisation of the allergen Sal k 1, a diagnostic marker for sensitisation to *Salsola kali*

Mas, S¹; Barderas, R¹; Boissy, P²; Monsalve, R²; Colás, C²; Rodríguez, R¹; Villalba, M¹

¹Biochemistry and Molecular Biology I, Complutense University of Madrid, Madrid, Spain; ²Research and Development Department, ALK-Abelló, Madrid, Spain; ³Hospital Clínico Universitario 'Lozano Blesa', Zaragoza, Spain

Background: Plants from *Chenopodiaceae* family are becoming important allergenic pollen releasers as the land desertification occurring in many countries is spreading these weeds and amplifying these allergies. The most relevant allergenic members of this family are *Salsola kali* and *Chenopodium album*. The marker of sensitisation to *S. kali* pollen is the major allergen Sal k 1, a highly polymorphic protein belonging to the pectin methylesterase enzyme family. The natural form of the protein is being used in diagnostic protocols as the ImmunoCAP ISAC or the Advia-Centaur immunoassay system. However, low amounts of the protein, many isoforms, and a rather cumbersome process of purification make difficult to obtain it from the pollen.

Objectives: To produce the recombinant Sal k 1 (rSal k 1) in *Escherichia coli* and to determine its biochemical and immunological equivalency with the natural protein isolated from *S. kali* pollen.

Methods: rSal k 1 was obtained in *E. coli* and purification achieved taking advantage of the histidine tag at the C-terminal end of the protein. Biochemical characterisation was performed by spectroscopic analysis and immunologically using monoclonal and polyclonal antibodies raised against nSal k 1 by ELISA and immunoblotting. The natural protein was used as reference. Eighty-nine sera from *S. kali* sensitised patients were used.

Results: rSal k 1 was purified to homogeneity by affinity chromatography using a Ni-NTA column with yields of about 5 mg/l of cell culture. Circular dichroism experiments confirmed that rSal k 1 was properly folded at secondary structure level. Immunological assays performed with monoclonal and polyclonal antibodies

revealed similar IgG binding for both proteins. The population of 89 patients' sera and monoclonal antibodies tested showed very close immunological behaviour for rSal k 1 and nSal k 1 in immunoblotting. However, certain variability of the IgE binding was observed between both proteins in ELISA, perhaps due to the involvement of conformational epitopes.

Conclusions: Recombinant and natural Sal k 1 showed similar immunological properties, indicating that rSal k 1 is able to cover most of the IgE epitopes of nSal k 1. Accordingly, rSal k 1 could be used as diagnostic tool in clinical protocols instead of the natural form due to its easy production and purification.

772

Prolins sensitisation is a marker of clinical risk in children affected by pollinosis?

Verini, M¹; Cingolani, A²; Nigro, R³; Scaparrotta, A³; Consilvio, NP²; Cerasa, M²; Di Pillo, S³; Chiarelli, F²; Molecular Allergology

¹Paediatric School of Medicine, University of Chieti, Chieti, Italy; ²Paediatric Department, University of Chieti, Chieti, Italy; ³University of Chieti, Chieti, Italy

Background: Profilins are gastrolabile and thermolabile proteins contained into eukaryotic cells of vegetables world involved in cellular movement processes. They are considered 'pan-allergens' because contained both in pollen of many allergen's source (grasses, pellitory, olive tree, birch, absinth and ambrosia) than in fresh and dry fruits (melon, tomato, pineapple, orange, apple, pear, banana, cherry, strawberry, peanuts, hazelnut and soy). While profilins' dangerous effects in adult is still discussed there are not yet studies about their effect in paediatric age.

Method: Sensitisation to profilins was studied in 295 children (mean age = 9.88 ± 3.69 years) affected by pollens' rhynitis and/or asthma (Skin Prick Test and specific IgE positive for grasses, pellitory and olive tree), by Specific IgE Immunocap against Phleum Pratense and Birch molecular allergenes Phl p12 and Betv2. A comparison between children with Phlp12 positivity and Phlp12 negativity has been made to analyse the differences in pattern of vegetables sensitisation and in incidence of vegetables ingestion related symptoms as SOA, urticaria or anaphylaxis.

Results: In our serie 68 children (23%) were sensitised to profilins (Phlp12 and Betv2) while 227(76%) were not. The group of profilins sensitised children showed these differences with not sensitised group:

1) Statistically higher specific IgE levels against wheat, tomato and peanuts;

2) Statistically higher extra-respiratory symptoms score (gastrointestinal: abdominal pain, vomit; cutaneous: urticaria; systemic: angioedema and anaphylaxis): 2.04 vs 1.65 ($P = 0.05$),

3) Statistically higher incidence of vegetables related symptoms (Oral Allergic Syndrome/ urticaria /angioedema /anaphylaxis): children with Phlp12 positivity = 22.4% vs children with Phlp12 negativity = 5.28% $X^2 = 8.9$, $P < 0.01$. No differences concerning asthma and rhinitis score were observed between the groups under study.

Conclusion: This study shows the relation between Phlp12 and Betv2 profilins sensitisation with the risk of allergic symptoms related to vegetables' ingestion in pollens sensitised children.

773

Proteomic tools for characterisation of allergen products

Spiric, J¹; Engin, A¹; Karas, M²; Vieths, S¹; Reuter, A¹

¹Division of Allergology, Paul-Ehrlich-Institut, Langen, Germany; ²University of Frankfurt, Institute of Pharmaceutical Chemistry, Frankfurt, Germany

Background: The most common causes of spring pollinosis in northern and central Europe are reactions toward pollen from trees of European white birch. Allergic diseases are diagnosed and treated using allergen extracts prepared from natural sources. Currently applied methods for characterisation of allergen products have limitations regarding the identification and quantification of allergens at isoform level in an extract (e.g. ELISA). Quantification of allergen content is one of the main aspects for product characterisation as major allergens effect the potency of allergen products. Therefore, the aim of this study was to develop a novel method capable of determining relative abundance of proteins in a single run, recording a quantitative protein profile and quantifying all relevant allergens and their isoforms in a sample.

Method: 2D-PAGE coupled with mass spectrometry (MS) was used to study isoform distribution in birch pollen extract, which gave the basis for development of a liquid chromatography (LC) -ESI-MS method capable of quantifying allergens, isoallergens, and recording quantitative protein profile.

Results: 2D-PAGE revealed the presence of five birch pollen allergens Bet v 1, Bet v 2, Bet v 4, Bet v 6, and Bet v 7. There were up to seven different isoforms of Bet v 1 identified per spot, and more than one spot was identified for other birch pollen allergens. Furthermore, the number and the type of allergens identified in 2D correlates

with allergens detected by label free MS. Quantitative values were obtained for all betula verrucosa allergens and several non allergenic proteins. This allows quantitative comparison of the entire protein profile of allergen products.

Conclusion: The present study indicates that this LC-MS^E method for characterisation and quantification of proteins in birch pollen provides a great perspective and appears to be a dependable tool that could be applied for identification and quantification of allergens and isoallergens in parallel. Our study suggests that this promising technology could replace current assays for allergen and protein profile detection in the field of allergology and especially in the field of allergen product characterisation. Moreover, this methodology could be applied to characterise also other protein based medicine products, and might contribute to enhance safety and efficacy of biomedicinal products.

774

Prevalence of skin sensitisation to LTP from peach: comparison between different ethnic groups

Carreño-Rojo, A¹; Mérida-Fernández, C¹; Ramírez-Hernández, M¹; Pajarón-Fernández, MJ¹; Huertas-Amorós, AJ¹

¹Allergy, Complejo Hospitalario Universitario de Cartagena, Cartagena Murcia, Spain

Background: The aim of this study was to compare the prevalence of skin sensitisation to LTP from peach (LTPp) between childrens and adults from different ethnic groups: Natives (Caucasian race), Maghrebians and South American people, residing in the Southeast of Spain.

Method: Native, Maghrebian and South American patients attending for the first time to our department from May 2011 to December 2012 suffering from respiratory/cutaneous symptoms or food allergy were included. Other ethnic group individuals were excluded. We divided patients into two groups: ≤12 years old and >12 years old. Skin prick tests with standard neoallergens and food allergens including LTPp (ALK- Abelló) were performed in all patients.

Results: The 2.226 individuals (53.7% females and 46.3% males, mean age 29.7 ± 17.7 year old) were included: 1937 (87%) natives, 148 (6.6%) Maghrebians and 141 (6.3%) South Americans. 455 (20.4%) patients were ≤12 years old and 1771 (79.6%) >12 years old. Skin prick test with LTPp was positive in 198 (8.9%) patients. Prevalence of skin sensitisation to LTPp depending on the age and ethnic group is showed in the table 1. Ethnic

group ≤ 12 years old Patients (n) /LTPp + (n/%) >12 years old Patients (n) /LTPp + (n/%) Natives 380/33 (8.7%) 1557/158 (10.1%) Maghrebians 35/2 (5.7%) 113/1 (0.9%) South Americans 40/2 (5%) 101/2 (2%) Total patients 455/37 (8.1%) 1771/161 (9.1%) Table 1 Statistical differences (P < 0.0001) in the prevalence of skin sensitisation LTPp between native, Maghrebian and South American adults were found but not in the group ≤12 years old.

Conclusion: Prevalence of skin sensitisation to LTPp is higher in native adults than in Maghrebian and South American. No differences were found in the prevalence of LTPp in younger patients of different ethnic groups. These results suggest that sensitisation to LTPp could be caused by environmental factors and/or dietetic habits in early childhood.

775

Expression and IgE reactivity of the fatty acid binding protein from the shrimp *Litopenaeus vannamei*

Puerta, L¹; Múnera, M¹; Pacheco, E¹; Martínez, D¹; Zak-zuk, J¹; Caraballo, L¹

¹Institute for Immunological Research, University of Cartagena, Cartagena, Colombia

Background: The allergenic properties of fatty acid binding proteins (FABP) from arthropods such as domestic mites and cockroaches have been demonstrated. The allergenic role of this highly conserved protein in other allergenic sources such as shrimp is unknown.

Method: The nucleotide sequence of FABP from shrimp *L. vannamei* (Lv FABP), (GenBank: ADK66280.1) was optimised for codon utilisation in *E. coli* expression systems and obtained by gene synthesis, then cloned into the expression vector and transformed into *E. coli* to produce the recombinant protein. Twenty eight allergics to shrimp were selected based on clinical history of allergy and positive skin test. IgE reactivity to shrimp allergenic extract and recombinant Lv FABP were determined by ELISA, in addition IgE reactivity to *Blomia tropicalis* extract and Blo t 13.0101 allergen were analyzed. Basophile activation assays were determined by flow cytometric in whole blood samples.

Results: The recombinant Lv FABP was obtained with N-terminal Hisx6 tag as inclusion bodies, requiring solubilization with urea followed by oxidative refolding. The purified protein showed a molecular weight of 16 KD. Frequency of positive IgE reactivity in sera from allergy subjects to shrimp was 25.0%, (7/28) and IgE levels ranging from 0.12 to 0.43 optical density. No IgE reactivity was detected in 13 sera

from non allergic subjects. All sera with positive IgE reactivity to Lv FABP, were also positive to the homologue allergen from *B. tropicalis* mite, Blo t 13.01.01. Basophile activation to recombinant Lv FABP, indicated by a stimulation index equal or greater than 2, was observed in two of three allergic individuals analyzed.

Conclusion: We report the obtaining of the recombinant FABP from *L. vannamei*, and demonstrated its IgE reactivity in shrimp allergy individual, this molecule represents a novel allergen from shrimp with homology to group 13 mite allergens.

776

Component resolved diagnosis: recording of discrepancies between single and multiplex IgE analysis

de Boer, D¹; Bons, JAP¹; Nieuwhof, CM²; Menheere, PPCA¹

¹Central Diagnostic Laboratory, Maastricht University Medical Centre, Maastricht, The Netherlands; ²Digestive Diseases Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands

Background: ISAC (Immuno Solid-phase Allergen Chip) is a multiplex assay used in Component Resolved Diagnosis (CRD). By measuring specific IgE against >100 allergen components in one single run a CRD profile is established using simply 20–30 µl of sample. Applying the ImmunoCAP assay also CRD can be achieved, but in one single run only one component can be analyzed using 40 µl of sample. In principal the manufacturer claims that the results of ImmunoCAP and ISAC techniques are standardised towards the WHO IgE 75/502 standard. However, as both techniques differ significantly, an equivalent performance requires more than standardisation. In this study we report discrepancies between ImmunoCAP and ISAC analysis for some allergen components as observed in our daily routine analysis.

Method: Patients (n = 86) suspected for food and/or insect allergy, for which CRD was applied because of diagnostic reasons, were included. ImmunoCAP and ISAC 103/112 analyses were performed according to the instructions of the manufacturer. Recording of discrepancies between identical allergen components were focused on selected milk (nBos d 8; casein), soy (rGly m 4; PR-10 protein) and wasp venom (rVes v 5; antigen-5) components. If required supplemental ImmunoCAP or ISAC analysis was performed. While the ImmunoCAP assay is a quantitative assay, the ISAC assay is a semi-quantitative assay. Therefore, ISAC results were interpreted in combination with its analytical uncertainty as established in a separate study. A

discrepancy was defined as a result that in the correlation of ImmunoCAP and ISAC results was out-side the 99% ISAC analytical uncertainty limit.

Results: With respect to rGly m 4 (three out of eight patients), nBos d 8 (7 out of 13 patients) and rVes v 5 (2 out of 9 patients) allergen components the discrepancies were 38%, 54% and 33%, respectively. For those discrepancies, the ISAC results were always significantly lower than that of ImmunoCAP. No consistent cause could be assigned. The observed differences may have a multifactorial background, although some differences are inherent to samples themselves.

Conclusion: In CRD and despite the limited number of observations, a significant number of discrepancies were observed between the results of rGly m 4, nBos d 8 and rVes v 5 ImmunoCAP versus ISAC analysis. ISAC gave in all of those cases considerably lower values. Understanding of the cause of these discrepancies is necessary for an adequate interpretation of ISAC results.

777

IgE to Der p 1 and Der p 2 as predictors of airway response to house dust mite

Minami, T¹; Fukutomi, Y¹; Taniguchi, M¹; Saito, A¹; Yasueda, H¹; Nakayama, S²; Tanaka, A²; Mitsui, C¹; Hayashi, H¹; Tsuburai, T¹; Maeda, Y¹; Mori, A¹; Hasegawa, M¹; Akiyama, K¹

¹Clinical Research Center for Allergy and Rheumatology, Sagami Hospital National Hospital, Sagami, Kanagawa, Japan; ²Phadia AB Thermo Fisher Scientific, Uppsala, Sweden

Background: Component-resolved diagnostics has recently been introduced into clinical practice. However, few data are available regarding the clinical usefulness of measuring IgE to allergen components from house dust mite (HDM) in the diagnosis of genuine HDM allergy. Bronchoprovocation challenge using allergen aerosol is considered to be a more specific indicator of allergen-specific airway reactivity than other allergy tests.

Objective: To evaluate the diagnostic efficiency of measuring the levels of IgE to allergen components from *Dermatophagoides pteronyssinus* (DP) as a predictor of immediate asthmatic response (IAR) after bronchoprovocation challenge.

Method: Sixty-five asthmatic patients who reported asthmatic symptoms upon house dust exposure underwent bronchoprovocation challenge using DP extract. The levels of serum-specific IgE to crude DP and nDer p 1, rDer p 2 and rDer p 10 in patients who showed IAR ($n = 41$) were compared with those in patients who showed no IAR ($n = 24$).

Results: The levels of IgE to crude DP, nDer p 1, rDer p 2 were significantly higher in patients showing IAR than in those not showing IAR (crude DP, $P < 0.01$; nDer p 1, $P < 0.01$; rDer p 2, $P < 0.01$). Receiver operating characteristics (ROC) analysis showed that the levels of IgE to nDer p 1 and rDer p 2 predict the outcome of a bronchoprovocation challenge similarly to IgE to crude DP (area under the ROC curve: crude DP, 0.953; nDer p 1, 0.949; rDer p 2, 0.945). The specificities of IgE to nDer p 1 and rDer p 2 were 67 and 75%, respectively, which were higher than that to crude DP (46%).

Conclusion: Specific IgE to nDer p 1 and rDer p 2 was an excellent and a more specific predictor of IAR. These findings validate the clinical usefulness of IgE to nDer p 1 and rDer p 2 as a diagnostic tool for genuine house dust mite allergy.

778

Immunoreactive recombinant Alt a 1 expressed in *Pichia pastoris*

Wünschmann, S¹; Koid, A¹; Lohman, M¹; Chapman, M¹
¹INDOOR Biotechnologies Inc., Charlottesville, VA, United States

Background: The three-dimensional structure of recombinant Alt a 1 expressed in *Escherichia coli* has recently been determined by X-ray crystallography (Chruszcz et al., JACI, 2012). However, this *E.coli*-expressed rAlt a 1 is no longer commercially available. Our goal was to produce rAlt a 1 in *Pichia Pastoris*, a eukaryotic system more closely related to *Alternaria alternata*.

Method: Recombinant Alt a 1 was expressed in *Pichia pastoris* and purified by gel filtration chromatography. *Pichia*-expressed rAlt a 1 was compared to *E.coli*-expressed rAlt a 1 by SDS-PAGE, CD spectroscopy, monoclonal antibody ELISA and chimeric-IgE antibody ELISA.

Results: SDS-PAGE of *Pichia*- and *E.coli*-expressed rAlt a 1 identified the Alt a 1 monomer at the expected molecular weight of ~15 kD as well as the dimer at 30 kD. Following gel filtration, *Pichia*-expressed Alt a 1 presented primarily as a dimer on non-reducing SDS-PAGE. The two forms of rAlt a 1 showed overall comparable CD spectra and similar reactivity in mAb- and IgE-Ab ELISAs. Specific IgE from all sera of *Alternaria alternata* allergic patients reacted to both forms of rAlt a 1 with excellent correlation ($n = 34$, $R^2 = 0.93$, $p < 0.001$).

Conclusion: *Pichia*-expressed Alt a 1 is almost identical to *E.coli*-expressed Alt a 1 in mAb and IgE binding assays. Alt a 1 produced in a eukaryotic system is a useful

reagent for diagnostic assays to determine sensitisation to *Alternaria*, and has other applications in T-cell studies, histamine release assays and mouse models of asthma.

779

Reproducibility analysis of multiplex IgE analysis

Martínez-Aranguren, RM¹; Lizaso, MT²; Goikoetxea, MJ¹; García, BE²; Cabrera-Freitag, P³; Trellez, O⁴; Sanz, ML¹; Research Network in Adverse Reactions to Allergens and Drugs (RIRAAF)

¹Department of Allergy and Clinic Immunology, Clínica Universidad de Navarra, Pamplona, Spain; ²Allergy Service, Complejo Hospitalario de Navarra, Pamplona, Spain; ³Department of Allergy and Clinic Immunology, Clínica Universidad de Navarra, Madrid, Spain; ⁴Department of Computer Architecture, Universidad de Málaga, Málaga, Spain

Background: The ImmunoCAP ISAC-CDR-112 (ThermoFisher, Uppsala, Sweden) is a fluoro-enzyme-immunoassay that allows detection of specific IgE to 112 molecular components from 51 allergenic sources. However, few studies of reproducibility have been carried out. We studied the reproducibility of this technique intra- and inter-assay, as well as interbatch- and interlaboratory-assay.

Method: Twenty samples were studied, nineteen sera from polysensitized allergic patients, and the technique calibrator, CTR02. The CTR02 is composed of known concentrations of specific chimeric antibodies to 15 allergens and is used to create the calibration curve of each new assay. We measured the sIgE from CTR02 and three patients' sera ten times in the same and in different assays. Furthermore, all samples were tested in two laboratories and with two batches of ISAC kit. To analyse the reproducibility and the agreement of the results obtained, we calculated the intraclass coefficient correlation (ICC) to each allergen and the coefficient of variation (CV) of the 15 allergens of the calibration curve. The level of agreement calculated by ICC, is expressed according to the classification of Fleiss: very good (ICC >0.91), good (0.91–0.71), moderate (0.71–0.51), poor (0.51–0.31) and bad (ICC <0.31). The CV is expressed in % as median and interquartile range.

Results: The agreement in the intra- and inter-assay analysis was very good to 94 allergens and good to one, whereas in the 17 allergen resting, the ICC could not be calculated because all their determinations were zero. In the interlaboratory analyse, we obtained a very good agreement to 73 allergens, good to 22, moderate to 6 allergens, and poor to two. And in the interbatch analyse, the agreement was very good to 82 allergens, good to 14, moderate

to 5, and poor and bad in one allergen. The CV in the determinations performed of sera from allergic patients were 9.9% (6.8–23.7), 27.1% (17.3–95), 20.3% (16–29.8) and 17.8% (12.3–34.2) in the intra- and inter-assay, interlaboratory and inter-batch analyse, respectively. Moreover, the CV% of the results obtained with CRT02 were slightly lower: 7.7% (6.3–9.1), 7.8% (5.7–15.6), 7.3% (5.9–9.5) and 6.8% (3.7–10.6), respectively.

Conclusion: The allergen microarray immunoassay, ISAC CRD112, is a reproducible *in vitro* diagnostic tool for determination of sIgE to a wide variety of allergens and these have a low variability in the results.

780

Clinical and IgE profiling of birch (*Betula verrucosa*) allergic individuals suffering from allergic reactions to raw fruits and vegetables

Tolkki, L¹; Alanko, K¹; Petman, L²; Skydtsgaard, MB³; Gronager, PM³; Seppala, U³; Ranki, A¹

¹Department of Skin and Allergic Diseases, Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland; ²Allergy Testing Unit, Department of Skin and Allergic Diseases, Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland; ³Global Research, ALK-Abell6, Horsholm, Denmark

Background: Birch pollen allergic individuals often report having mucosal symptoms after consuming raw fruits and vegetables. The aim of the present study was to assess clinical and specific IgE profiles in individuals suffering from birch pollen allergy and concomitant immediate type allergic reactions to raw fruits and vegetables.

Methods: A total of 59 adults with clinical and skin prick test (SPT) confirmed birch pollen allergy were included in the study. All subjects were interviewed using a structured questionnaire and examined *in vivo* by an open challenge test, applying the appropriate fruit and vegetable(s) crushed on ventral side of arm skin and/or with labial challenge. ImmunoCAP and ImmunoCAP ISACTM were used as *in vitro* diagnostics to demonstrate IgE based sensitisation profiles for each individual and principal component analyses (PCA) were used to analyses of the IgE data sets.

Results: Out of 59 birch allergic individuals 54 (92%) had positive prick-prick test with raw potato, carrot, apple and/or hazelnut and the SPT was always positive when the corresponding skin challenge was defined positive. The majority 67% (36/54) reported history of immediate skin contact symptoms such as itching, redness or urticarial reactions when handling raw potato, carrot, apple and/or hazelnut. Although the ImmunoCAP ISACTM inhibition assays

with rMal d 1 and rBet v 1 demonstrated that Bet v 1 is driving the sensitisation against pathogenesis related -10 (PR-10) proteins, the PCA demonstrated that specific IgE raised against pollen allergens could not be used to predict *in vivo* sensitisation to fruits and vegetables.

Conclusions: Among the birch allergic individuals allergic reactions to fruits and vegetables are not limited to the oral mucosa. In our study most of the individuals reported and showed immediate skin symptoms when handling raw fruits and vegetables and/or challenged with appropriate food. The present study also shows that component based IgE profiling does not enhance the diagnostic potential in case of pollen-food syndrome. Careful clinical characterisation of allergic individuals as well as identification of additional sensitising allergens is still needed to identify individuals suffering from pollen related food allergies. Misdiagnosis of food allergies may lead to unnecessary avoidance diets which may result in impaired quality of life of affected individuals.

781

Comparison of two methods for determination of allergen-specific IgE in a population sample of Asian children: PATCH study

Chang, S-W¹; Tu, Y-L^{2,3}; Tsai, H-J^{4,5}; Huang, J-L^{2,3}; Yao, T-C^{2,3}

¹Clinical Informatics and Medical Statistics Research Center, Chang Gung University College of Medicine, Taoyuan, Taiwan; ²Division of Allergy, Asthma, and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan; ³Department of Pediatrics, Chang Gung University College of Medicine, Taoyuan, Taiwan; ⁴Division of Biostatistics and Bioinformatics, Institutes of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan; ⁵Feinberg School of Medicine, Department of Pediatrics, Northwestern University, Chicago, IL, United States

Background: There has been growing interest in the application of microarrays for the measurement of allergen-specific immunoglobulin E (IgE) in the past few years. Specifically, BioIC[®], a recently developed microfluidic microarray immunoassay, permits simultaneous determination of specific IgE to multiple allergens in a single step using small amount of serum, at a relatively low cost. This study aimed to assess the performance of BioIC in comparison with that of a well-established assay, ImmunoCAP[®], in a population sample of Asian children.

Methods: An inter-assay comparison of BioIC[®] AD40 panel and ImmunoCAP[®] was performed using serum samples from a population-based sample of 190 atopic children aged 5–18 years participating in the Prediction of Allergies in Taiwanese

Children (PATCH) study. We compared the performance of the two assays for detecting specific IgE to twelve major inhalant and food allergens (*Dermaphagoides pteronyssinus*, German cockroach, dog dander, cat dander, egg white, milk, codfish, shrimp, crab, wheat, peanut and soybean).

Results: A total of 2280 paired assay results were analyzed. High agreement was observed between the results of both assays for detecting allergen-specific IgE, with an average total agreement rate of 81.5% (95% CI: 76.0–87.0%), ranging from 66.3% (95% CI: 59.6–73.0%) for egg white to 96.3% (95% CI: 93.6–99.0%) for soybean. The one-class agreement rate ranged from 60.5% (95% CI: 53.6–67.5%) for *D. pteronyssinus* to 99.5% (95% CI: 98.4–100.0%) for soybean, with an average of 92.9%.

Conclusion: These data demonstrate high agreement between the results of BioIC[®] and ImmunoCAP[®] in a population-based setting, indicating that BioIC[®] may serve as an accurate and cost-effective alternative for multiplexed determination of allergen-specific IgE.

782

Validation of an ELISA method for the quantification of Amb a 1

Arilla, C¹; Ibarrola, I¹; Brena, S¹; Zamarreño, J¹; Martínez, A¹; Asturias, JA¹

¹Bial Aristegui, R&D, Zamudio, Spain

Background: Amb a 1, a pectate lyase of 38 kDa, is the *Ambrosia artemisiifolia* most relevant allergen, since 95% of ragweed-sensitive individuals react to this protein in skin tests and show high serum IgE antibody titers. Allergen manufacturing process should involve the measurement of this relevant allergen in order to maintain its level in the freeze dried pollen extracts. The aim of this study was to validate a double phase ELISA to quantify Amb a 1 according the Guideline of Validation of Allergen Products: Production and Quality Issues^{*} (CHMP/BWP/304831/2007) and to confirm the manufacturing process consistency.

Method: Amb a 1 ELISA kit (Indoor Biotechnologies Ltd) was used following the manufacturer's instructions. It was based in rabbit polyclonal IgG antibodies raised against short ragweed allergen Amb a 1 purified by affinity chromatography to capture, and the same biotinylated antibodies for detection. The major allergen concentration of the *A. artemisiifolia* extracts was measured by interpolation in the linear portion of an Amb a 1 standard curve (62.5–1 ng/ml) in the validation assays.

The parameters studied were repeatability, intermediate precision, accuracy and limit of quantification. Amb a 1 content was tested in five batches of the freeze-dried *Ambrosia artemisiifolia* pollen extract from three different pollen suppliers.

Results: The study meets the requirement of repeatability since the coefficient of variation (CV) of the percentages of Amb a 1 calculated from 9 curves of the extract and performed under the same operating condi-

tions by two different analysts was less than 8.5%. In the intermediate precision, neither different analysts nor different days of analysis had a significant effect on the result. The method had the required level of accuracy because t_{exp} 1.502 was lower than the t_{tab} . The limit of quantification of the *A. artemisiifolia* pollen extract was 83 ng/ml which meets the requirements of precision (CV = 12.8%) and accuracy (relative error = 0.9%). The content of the rel-

evant allergen Amb a 1, was similar in all the batches, and fall between 73% and 100% of the IHRP content which are much less than allowed by the Eur. Ph. (50–200%).

Conclusion: The two-step ELISA method is suitable for quantifying Amb a 1 because it provides consistently and repeatedly results that meet the established specifications.

Poster Session 23

Molecules, chips and cells: new tools in allergy diagnosis

784

Effect of cryopreservation, cryostorage and thawing on mononuclear cell populations: detailed examination of CD4⁺ T cell subsets

Collier, F^{1,2}; Tang, M^{3,4}; Southall, C^{1,2}; Kennedy, D^{1,2}; Vuillermin, P¹; BIS Investigator Group
¹Barwon Health, Child Health Research Unit, Geelong, Vic., Australia; ²Barwon Health, Barwon Biomedical Research, Geelong, Vic., Australia; ³Murdoch Childrens Research Institute, The Royal Children's Hospital, Parkville, MO, United States; ⁴The University of Melbourne, Melbourne, Vic., Australia

Background and objective: Samples of blood mononuclear cells (MNC) are often cryopreserved for extended periods of time prior to analysis. This allows them to be batch analysed at a later date for both phenotypic and functional studies. While cryopreservation is a useful tool, it is not known whether it can affect cell function or distribution of cell phenotypes. We aimed to determine whether cryopreservation, storage and thawing altered MNC populations of lymphocytes, including T cells, subsets CD4⁺ and CD8⁺, CD4⁺ recent thymic emigrants (RTE) and regulatory T cells (Treg).

Method: MNC collected from infants participating in the Barwon Infant Study were isolated from cord blood. Small numbers of cells were stained with a panel of antibodies and remaining MNC cryopreserved in liquid nitrogen. Selected samples were then thawed and stained with the same panel of antibodies, re-analysed by flow cytometry and compared to freshly isolated MNC. Cell number and viability was determined using Trypan Blue. Data was compared using Wilcoxon matched-pairs signed rank test.

Results: Cells were frozen for periods ranging from 1 to 12 months. After thawing 60 ± 6% of the MNC cells were successfully retrieved with 93 ± 1% viability (mean ± sem, *n* = 11). Flow cytometric analysis of the CD4⁺ T cells showed a significant decrease in CD25⁺ T helper cells (activated phenotype) (*P* = 0.01), however, measures for the remaining T cell populations: T cells (CD3⁺), T cell subsets (CD4⁺, CD8⁺), naïve CD4⁺ T cells (CD45RA⁺), CD4⁺ Recent Thymic Emigrants (CD45RA⁺, CD31⁺) were unchanged. In addition, using both surface (CD127^{low}) and intracellular (FoxP3⁺)

markers there was no change in the percentage of Treg subsets.

Conclusion: In comparison to results obtained from fresh cord blood samples, cryopreservation, storage and thawing was associated with a reduction in CD25⁺ activated T helper cells, but no change in the proportion of other T cell subsets including the specialised immune suppressors, Tregs. These results indicate that, in our laboratory, the use of cryopreserved cells will yield similar cell populations to freshly processed cells.

785

Type I drug allergy: comparison of two basophile activation tests

Micaletto, S¹; Ballmer-Weber, B¹; Schmid-Grendelmeyer, P¹; Petrusch, U¹
¹Allergy, University Hospital of Zurich, Zurich, Switzerland

Background: In case of suspected IgE dependent drug reactions, skin testing is most commonly used to establish the diagnosis of drug hypersensitivity. Sensitivity of skin testing is low especially for drugs not belonging to beta-lactam antibiotics. Flow CAST[®] can be performed in addition to skin testing as *in vitro* diagnostics. The test is intended to detect basophil activation in response to the culprit drug by flow cytometry. Until now the marker combination CCR3 and CD63 was used by Flow CAST[®] to measure activated basophils. To increase sensitivity of *in vitro* basophil activation testing, Flow CAST[®] highsens, was established. Activated basophils are now measured by CCR3, CD63 and CD203c.

Method: In this study 21 consecutive patients with clinically suspected type I allergic reaction were included. Flow CAST[®] and Flow CAST[®] highsens were performed with the culprit drug and compared. In all patients skin tests were performed. We tested NSAIDs, muscle relaxants and antibiotics.

Results: Activated basophils in the reaction to the culprit drug were found in 12 of 21 patients. In 9 of these positive results only the Flow CAST[®] highsens was positive, while the regular Flow CAST[®] was negative. In two patients the regular test was positive with a negative result in the

highsens test. Surprisingly, all patients with a positive Flow CAST[®] highsens had a negative skin test for the culprit drug. We also found positive skin tests in two patients with negative *in vitro* tests.

Conclusion: Our data suggest a substantial higher sensitivity of that Flow CAST[®] highsens when compared to the regular Flow CAST[®]. From the *in vitro* data we would have to estimate a sensitivity of approximately 50%. *In vitro* tests should be combined with the skin tests to maximise the sensitivity. To definitely determine the sensitivity of Flow CAST[®] highsens comparisons with drug provocation tests in prospective clinical trials should be addressed.

786

A quantitative ELISA for determining Antigen 5 content in wasp venom

van Deursen, D¹; van den Hout, R¹; van Ree, R²; Kerkvliet, E¹

¹Development, HAL Allergy BV, Leiden, The Netherlands; ²Academic Medical Center, Experimental Immunology, Amsterdam, The Netherlands

Background: Allergy induced by wasp venom can be treated by means of allergen-specific immunotherapy. For wasp venom, the main allergen reported is the protein Antigen 5, known by the allergen nomenclature as Ves v 5 (for *Vespula vulgaris*) or Ves g 5 (for *Vespula germanica*). We developed a major allergen ELISA and investigated the Antigen 5 content in different wasp venom products.

Method: 96-well plates are coated with monoclonal antibodies directed against Ves g 5. After blocking, dilution ranges of wasp venom preparations are added and detection is done with a polyclonal antibody from rabbit directed against Ves g 5. Subsequently, incubation is performed with an HRP-conjugated goat-anti-rabbit antibody. The 96-well plates are stained with TMB and the concentration Antigen 5 is calculated by the use of the standard curve. The new method uses the four-parameter non-linear regression model for determining the Antigen 5 content in test preparations. The standards used in the ELISA is Antigen 5 protein purified from wasp venoms, which was characterised and quantified by amino acid analysis, FPLC, SDS-

PAGE and protein determination. Antigen 5 concentration was determined in one drug product and three different suppliers of raw materials.

Results: The standards showed good four-parameter dose response curves with high correlations ($r > 0.99$) and the samples tested showed high intra- and inter assay precision ($CV \leq 8\%$). The Antigen 5 content of the wasp venom batches tested was: 1.8% (drug product; *Vespula spp.*), 1.8% (supplier A; *Vespula spp.*), 1.7% (supplier B; *Vespula spp.*), 1.9% (supplier C; *Vespula vulgaris*) and 2.0% (supplier C; *Vespula germanica*). This is in accordance with data from the literature.

Conclusion: The ELISA method is a suitable technique to quantify Antigen 5 content in different wasp venom products (raw materials and drug product).

787

Defining the optimal cut-off values for flow-assisted basophil activation test in children with respiratory allergy to Dermatophagoides pteronyssinus

Gregorius, A¹; Czarnobilska, E²; Spiewak, R¹

¹Department of Experimental Dermatology and Cosmetology, Jagiellonian University Medical College, Krakow, Poland; ²Department of Clinical & Environmental Allergology, Jagiellonian University Medical College, Krakow, Poland

Background: To ensure the quality, bioequivalence and comparable performance of diagnostic allergy tests, their cut-off values should be established individually for each particular allergen and population in question. Here, we demonstrate the process of searching for the optimal parameters in case of testing for house dust mite allergy in children and adolescents.

Method: The study involved 60 children and adolescents with respiratory allergic disease (asthma and/or allergic rhinitis) divided into 2 groups: 30 patients with confirmed, clinically relevant D. pteronyssinus allergy (qualification for specific immunotherapy following EAACI guidelines served here as the gold standard) and 30 sex and age-matched controls with respiratory allergy to seasonal allergens (birch, timothy). Values of the COV most effectively separating children sensitised to D.p. from those non-sensitised were established based on the receiver-operator curve (ROC) analysis for a range of variables, including basophil stimulation at 5 allergen concentrations ranging from 22.5 ng/ml down to 0.00225 ng/ml, and area under curve (AUC) calculations made in attempt to combine two or more raw results in one parameter. The CD63-based commercial kit Flow2CAST together with commercial D.p. allergen (Buhlmann) was selected for

this study, as the only certified for *in vitro* diagnosis (IVD) that was available at the start of this study.

Results: The best COV in the analysed group was 9.76% activated basophils (allergen response minus background) at D.p. allergen concentration of 2.25 ng/ml (98.3% correctly classified cases), followed by the COV of 18.01% at 22.5 ng/ml (96.7% correctly classified cases), and AUC2 combining results for 22.5 ng/ml with 2.25 ng/ml (COV of 14.23% activated basophils) and AUC3 combining results for 22.5 ng/ml, 2.25 ng/ml, and 0.225 ng/ml (COV 15.14%) – each 96.7% correctly classified cases. The sensitivity/specificity rates were respectively: 96.7% and 100%, 100% and 93.3%, and each 100% and 93.3%.

Conclusion: Using the Flow2CAST, a single measurement of basophil stimulation with allergen at 2.25 ng/ml seems most efficient and economical for the confirmation or exclusion of respiratory allergy to Dermatophagoides pteronyssinus in children.

788

Possible use of the basophil activation test as a biomarker for allergen immunotherapy

De Amici, M¹; Benzo, S¹; Labò, E¹; Caimmi, S¹; Caimmi, D¹; Marseglia, A¹; Torre, C¹; Marseglia, GL¹

¹Pediatrics, University of Pavia- IRCCS Policlinico San Matteo, Pavia, Italy

Background: Allergen specific immunotherapy (AIT) is the only treatment able to act on the causes and not merely on the symptoms of allergy. AIT may mainly be administered in two forms, subcutaneous (SCIT) and sublingual (SLIT). Several trials have highlighted the efficacy and safety of both forms in allergic rhinitis and asthma, but no study has provided a definitive biomarker that may allow monitoring the clinical course of patients and demonstrating from both a biological and clinical point of view the effectiveness of the therapy. The aim of the present study was to evaluate the utility of the basophil activation test (BAT) as a biomarker for AIT effectiveness.

Method: We compared BAT in the diagnosis of graminaceae allergy with skin tests and measurement of specific IgE and we investigated whether basophils sensitivity can predict the outcome of AIT. A prospective study has been designed for the follow-up of allergic patients undergoing SLIT therapy. A retrospective study has been also designed to investigate a possible correlation between basophils sensitivity and clinical symptoms severity.

Results: 11 patients have been enrolled for the prospective study, eight with seasonal allergic rhinoconjunctivitis and three with asthma. BAT was carried out before the beginning of AIT (T0) to set the specific basal sensitivity of the patients' basophils. BAT has been repeated yearly for the entire duration of the therapy. For the retrospective study, 20 patients have been enrolled, 13 with seasonal allergic rhinoconjunctivitis and seven with asthma. These patients lack the evaluation of BAT at T0, and they were only tested during therapy. BAT results in both groups will be compared to clinical symptoms and specific IgE measurements. We also tested the evolution of BAT in 10 allergic patients, who didn't undergo allergen specific immunotherapy, as control group.

Conclusion: The aim of this study is to understand if BAT is a useful biomarker of AIT. This would allow evaluating safety and efficacy of the therapy in order to possible decide to continue or stop the prescribed immunotherapy. Our results might also help us identify different subgroups of patients that could show a good or bad response to AIT.

789

Phleum pollen sensitisation pattern for major allergen components in Southern Romania grass pollinosis

Vieru, M¹; Popescu, F-D¹; Tudose, AM¹; Dumitrescu, NV²

¹Allergology, University of Medicine and Pharmacy 'Carol Davila', Bucharest, Romania; ²Allergology, Hospital 'Nicolae Malaxa', Bucharest, Romania

Background: In the Romanian plain, sensitisation to grass pollen is higher compared with other pollens significant as aeroallergens. Timothy grass pollen is a source of important major allergens: Phl p 1 and Phl p 5.

Method: We collected sera of patients from the south part of Romania with a history of seasonal pollen allergy (presenting symptoms of rhinoconjunctivitis with or without asthma) in the grass flowering period of the region and positive skin prick tests to grass pollens, with or without sensitisation to other plant pollens with partial temporal overlap for their pollination season. Specific IgE antibodies against recombinant *Phleum* allergen components (Phl p 1, Phl p 5, Phl p 7, Phl p 12) were detected using a novel multiparameter immunoblot test system, based on single purified allergen components, SPAC Pollen1.

Results: From eighteen adult and children patients with positive skin prick tests to *Phleum pratense* pollen, 94.44% were sensitised to Phl p 1 beta-expansin and/or Phl p

5 allergen components. Sensitisation to Phl p 7 and Phl p 12 cross-reactive allergen components was less significant.

Conclusion: Specific IgE antibodies against major specific allergen components Phl p 1 and Phl p 5 detected by SPAC Pollen 1 can be used as a marker to genuine grass pollen allergy, important in the decision for allergen-specific immunotherapy.

790

Development of a multiplex assay for the determination of specific and total IgE and IgG4 for 65 different targets

Feizkhanova, GU¹; Filippova, MA¹; Talibov, VO¹; Demetieva, EI¹; Fooke-Achterrath, M²; Zasedatelev, AS¹; Rubina, AY¹

¹Russian Academy of Sciences, Engelhardt Institute of Molecular Biology, Moscow, Russia; ²DR. FOOKE Laboratorien GmbH, Neuss, Germany

A promising technique for modern allergy diagnostic is a biological microchip providing a multiplexed, highly sensitive quantitative assay. This system allows the simultaneous determination of different antibodies of different subclasses to multiple allergens in a single assay format. The AllergoChip, a biochip – an array of gel elements containing a large set of immobilised allergens – allows the determination of IgE and IgG4-antibodies in a single test using a minimal sample volume. The AllergoChip is manufactured by photoinduced copolymerization of different molecular probes with gel-forming monomers resulting in the formation of three-dimensional hydrogel elements (1 nl gel drops), a technology invented at the Engelhard Institute of Molecular Biology, Russian Academy of Sciences.

In the present setup the AllergoChip for the simultaneous assay of sIgE and sIgG4 for 65 allergens and total IgE and IgG4 contains different groups of allergens, recombinant allergens, extracts and antibodies to IgE and IgG4. The three dimensional hydrogel allows for native conformation and full accessibility of all epitopes. During the serum incubation specific complexes of sIgE and sIgG4 to the allergens in the gel are formed. The immune complexes are visualised using a mixture of anti-IgE-Cy5- and anti-IgG4-Cy3-conjugates and concentrations of sIgE and sIgG4 for specific allergens are determined simultaneously on the basis of the recorded fluorescence signals. In addition, concentration of total IgE and IgG4 is determined in the same assay. The AllergoChip is read out using the fluorescence Chip analyzer and the interpretation is performed using the *ImageAssay* software (Engelhard Institute of Molecular Biology),

which allows simultaneous analysis of two-wavelengths fluorescence images.

Comparative parallel assay of serum samples using AllergoChip and REAST method (ALLERG-O-LIQ, Dr. Fooke Laboratorien GmbH) showed good correlation between the two methods (correlation coefficients 0.89–0.92).

The AllergoChip will be further developed since the format of a biochip provides the unlimited increase of the number of allergens.

791

Molecular profiles of specific IgE to individual allergens of *Phleum pratense* in Spain

Beitia, JM¹; López-Matas, MA²; Vega, A¹; Alonso, A¹; Cárdenas, R²; Mateo, B¹; Carnés, J²
¹Allergy Section, Hospital Universitario de Guadalajara, Guadalajara, Spain; ²Laboratorios LETI, R & D, Tres Cantos, Spain; ³Allergy Unit, Hospital de Ciudad Real, Ciudad Real, Spain

Background: Advances in molecular biology have allowed the use of a large spectrum of purified allergens for routinely diagnostic purposes. Component Resolved Diagnosis (CDR) is a tool that characterises each patient's IgE antibody (sensitisation) profile to individual allergen components. The objective of the study was to determine the molecular profile of sIgE to individual allergens of *P. pratense* in a population from the centre of Spain.

Method: A population of 137 individuals from Guadalajara (centre of Spain) was included in the study. All of them were previously skin prick tested with a standardised extract of grasses (Laboratorios LETI SL). Patients with positive wheal size (>7 mm²) were included in the study and serum sample obtained. Specific IgE to a mixture of grasses and *P. pratense* allergens (Phl p 1, Phl p 2, Phl p 4, Phl p 5b, Phl p 6, Phl p 7, Phl p 11 and Phl p 12) were measured by CAP.

Results: Most patients were sensitised to Phl p 1 (beta expansin) (96.4%) and Phl p 4 (berberine bridge enzyme) (91.2%). The lower percentage was obtained for Phl p 7 (calcium-binding protein, polcalcin) (8%). One patient was negative to all allergens tested (0.7%). Average values for the specific IgE ranged between 23.0 kU/l for Phl p 5b (ribonuclease) and 4.2 for Phl p 12 (profilin). Thirty one different molecular profiles were found in the studied population.

Conclusion: Phl p 1 and Phl p 4 were the allergens with higher prevalence of sensitisation which is surprising in comparison with other studied populations. The less frequent was Phl p 7. The highest values for the specific IgE were obtained with Phl

p 5b, Phl p 1 and Phl p 7. There are a great variety of different profiles that could hamper the SIT with individual allergens.

794

Usefulness of molecular diagnosis

Caimmi, S¹; de Amici, M¹; Trovama, V¹; Caimmi, D¹; Testa, G¹; Marseglia, A¹; Licari, A¹; Marseglia, G¹
¹Pediatrics, University of Pavia- IRCCS Policlinico San Matteo, Pavia, Italy

Background: Molecular diagnosis (MD) has started a new era, but it is important to understand the clinical utility of the available tests. Since the appearance of molecular analysis in allergy, a systemic study of the main allergens has been performed, including the evaluation of possible cross-reactivity processes involved in allergic reactions. These new forms of component-resolved diagnosis reveal the antibody reactivity profile of allergic patients and highlight the specific molecules involved in the allergic diseases. The aim of the present study was to evaluate the diagnostic/therapeutic utility of molecular diagnosis in patients allergic to common allergens.

Method: Two hundred and five patients were enrolled in the study: 54 patients presented with allergic rhinitis or asthma (diagnosed on the basis of the ARIA and GINA guidelines), 56 patients with cutaneous signs (CS) and 95 patients with food allergy (FA). We compared the medical history and the results of skin prick tests (SPTs) with their pattern of molecular sensitisation through microarrays in order to analyse the clinical and therapeutic utility of MD in confirming the allergic disease. Serum specific IgEs were measured by the ImmunoCAP ISAC[®] in a microarray image analysis and expressed by Standardised Units for specific IgE (ISU-E).

Results: Medical history, SPTs and ISAC were in agreement in 60% of the patients. Considering the different diagnosis, in the 95 patients with FA, ISAC resulted to be a good diagnostic tool (80% of agreement), and a helpful tool for a therapeutic approach. ISAC results were extremely consistent (99%) with medical history and SPTs in patients presenting with severe forms of FA. This finding was less consistent in patients presenting with respiratory or cutaneous symptoms, with the exception of those patients presenting respiratory food-pollen related allergies (53%). On the other hand, it has to be underlined that in 10% of patients with FA, molecular findings were in contrast both with clinical history and SPTs.

Conclusion: The present study shows that MD has good diagnostic and monitoring

value in those patients presenting with severe forms of FA but it is also helpful in pts with food-pollen related allergy and gives the pattern of possible cross-reactivity between pollen and food. Further investigations will be necessary in order to detect the proper indication for allergen-specific immunotherapy before and after performing a MD in allergic patients.

795

Molecular diagnosis in dog allergy

Uriarte, SA¹; Sastre, J¹

¹Allergy Department, Fundación Jiménez Díaz, Madrid, Spain

Background: Allergy to dog is a frequent cause of rhinitis, asthma or contact urticaria. Dander, saliva and urine are sources of dog allergens. Currently, the prevalence of sensitisation to different dog allergens is not well known.

Objective: To measure specific IgE to different dog allergens.

Method: The pattern of IgE sensitisation to dog allergens, Can f 1, Can f 2, Can f 3, and Can f 5 was evaluated in 172 patients sensitised to dog. ImmunoCAP[®] and microarray ISAC[®] (ThermoFisher Scientific, Sweden) was used to measure specific IgE. A value >0.35 kU/l or >0.3 ISU was considered as positive for ImmunoCAP or ISAC respectively. Skin prick test was performed with an ALK-Abelló extract (Denmark).

Results: Specific IgE to Can f 1 was found in 66% (115) of patients, followed by Can f 5 in 33% (57), Can f 2 in 18% (31) and Can f 3 in 9.3% (16). Of note, a considerable amount of patients were monosensitised to Can f 5 (15% $n = 26$). The wheal diameters of skin test with commercial extract of dog were smaller in patients monosensitised to Can f 5. From a clinical point of view, most patients sensitised to any dog allergens referred symptoms when exposing to dogs.

Conclusion: In this study we show different pattern of sensitisation to dog allergens that are commercially available. Interestingly, a high prevalence of monosensitisation to Can f 5 was demonstrated. Can f 5 is an arginine esterase or prostatic kallikrein that is found only in male dogs. This finding could be of clinical interest because may explain differences in development of symptoms when exposed to male or female dogs. Further studies are necessary to establish if different patterns of sensitisation to dog allergens may have clinical consequences and response to immunotherapy.

796

Use of recombinant hymenoptera venom molecular allergen for specific IgE detection

Chuang, T¹; Bogdanovic, J¹; Bocsusis, N¹; Ruivo, J¹; Kubota, H¹; Sinha, S¹; Jaggi, K¹; Timmons, M¹; Hovanec-Burns, D¹

¹Siemens Healthcare Diagnostics Inc., Tarrytown, NY, United States

Background: IgE-mediated allergic reactions to insect venoms from the order Hymenoptera presents a serious anaphylactic risk for sensitised individuals. Studies using major and minor Hymenoptera allergens have been limited due to the number of component allergens available for analysis of specific IgE to both honey bee venom (HBV) and yellow jacket venom (YJV). In this study, we measure the allergen specific IgE profiles of suspected Hymenoptera allergic individuals using the following recombinant and synthetic components from HBV and YJV: phospholipase A₂ (rApi m 1*), hyaluronidase (rApi m 2*), acid phosphatase (rApi m 3*), melittin (sApi m 4*), phospholipase A_{1B} (rVes v 1*), and antigen 5 (rVes v 5*).

Methods: rApi m 1, rApi m 2, rApi m 3, rVes v 1, and rVes v 5 allergenic proteins were expressed in Sf9 insect cells, purified, and conjugated to biotin. Biotinylated sApi m 4 peptide was prepared using a peptide synthesiser. Sera from 18 subjects with a clinical history of venom allergy or known specific IgE reactivity to whole extract HBV or YJV allergens were tested for IgE to the component venom allergens using the IMMULITE[®] 2000 3gAllergy[™] Specific IgE assay. Assessment of the specificity of reactivity to HBV and YJV component reagents was determined by inhibition testing.

Results: Out of the 18 samples examined, 10 demonstrated IgE sensitivity to both HBV and YJV whole extracts. Three of the 10 double sensitised samples showed non-detectable (ND) IgE results to rVes v 1 and rVes v 5. On further examination of the 3 ND samples, 2 were found reactive to rApi m 2 while the third had low specific IgE to YJV extract (0.29 kU/l). All reactivity to HBV and YJV component allergens was confirmed by >70% signal inhibition in the presence of homologous inhibitors.

Conclusions: Detailed examination using HBV and YJV components allergens gives more information to help distinguish cross reactivity or true IgE sensitisation when results are confounded by clinical history or double sensitivity to whole extracts.

*For Research Use Only. Not for use in diagnostic procedures.

797

Correlation between specific IgE to single allergen components of timothy pollen and clinical expression of allergy to grass pollen

Savi, E¹; Peveri, S¹; Dell'Albani, I²; Incorvaia, C³; Frati, F²

¹Allergy Department, G. Da Saliceto Hospital, AUSL Piacenza, Piacenza, Italy; ²Medical and Scientific Department, Stallergenes Italy srl, Milan, Italy; ³Allergy/Pulmonary Rehabilitation Unit, ICP Hospital, Milan, Italy

Background: The currently available component resolved diagnosis (CRD) allows to measure the specific IgE (sIgE) to the single components of an allergen source. We evaluated the possible correlation between the level of sIgE to the different components of timothy grass (*Phleum pratense*) and the clinical data of respiratory allergy to grass pollen.

Method: One hundred forty patients with grass pollen allergy were included in the study. All patients were clinically classified according to ARIA guidelines for allergic rhinitis (AR) and to presence or absence of asthma. sIgE to Phl p 1, Phl p 5, Phl p 7, and Phl p 12 from *P. pratense* were measured by CAP System ThermoFisher. The correlation between the ARIA stage of AR and the presence of asthma was analyzed by the Spearman Rank test; the correlation between the clinical data and the levels of sIgE to single allergen components was analyzed by multivariate logistic regression concerning IgE and ARIA stage, while univariate logistic regression was used for IgE and asthma due to the dichotomic classification of asthma as present or absent.

Results: Clinically, 10 patients had intermittent AR, 48 had mild persistent AR, and 82 had moderate-severe persistent AR. Asthma was present in 86 patients and absent in 54. The correlation was significant ($P < 0.01$) for moderate-severe persistent AR and presence of asthma. The mean level (SD) of sIgE to the single allergen components were 23.41 (29.46) kU/l for Phl p 1, 16.04 (25.57) for Phl p 5, 0.55 (2.42) for Phl p 7, and 3.04 (8.46) for Phl p 12. The only significant correlation was found for low values of sIgE to Phl p 5 and absence of asthma ($P < 0.01$).

Conclusion: These findings confirm the clinical association between more severe AR and occurrence of asthma. No correlation was found between sIgE levels to single allergen components and ARIA stage of AR, while the significant correlation between absence of asthma and low values of sIgE to Phl p 5 suggests a possible use as surrogate marker of this parameter.

798

Allergenic molecules as markers in epidemiology – Italy macro-regional prevalence

Mari, A¹; Zuzzi, S¹; Santoro, M¹; Ferrara, R¹; Bernardi, ML¹; Zennaro, D¹; Alessandri, C¹; Dell'Albani, I²; Palazzo, P¹; Rapaiani, C¹; Incorvaia, C³; Frati, F²
¹Center for Molecular Allergology, IDI-IRCCS, Rome, Italy; ²Medical and Scientific Department, Stallergenes Italy srl, Milan, Italy; ³Allergy/Pulmonary Rehabilitation Unit, ICP Hospital, Milan, Italy

Background: Current epidemiology of allergic sensitisation to inhalants is still based on the use of allergenic extracts by mean of skin testing or singleplex IgE detection.

The recent introduction of both allergenic molecule preparations and the microarray technology allows us to undergo multiplex testing of each patient, using allergens as markers, and performing the testing in a routine fashion.

The aim of this preliminary study was to show how the above reported combination supported by an innovative information technology infrastructure (based on the Allergome platform) might set an innovative manner of studying allergic sensitisation epidemiology.

Method: All patients referred to the Center for Molecular Allergology, IDI-IRCCS, Rome, Italy, were tested using the ISAC microarray starting in 2006 up to 2012. Several versions of the ISAC have been used, bearing an increasing number of molecules from 76 to 112. Demographical and clinical data were collected during the first consult by a certified allergist.

Results: Collected data have been divided according to 5 Italian macro-regions (north-western, north-eastern, center, southern, islands). Depending on the allergen marker considered, tested subjects ranged from 30,550 (Phl p 4) to 58,033 (Bet v 1, Phl p 1). The Italian distribution of IgE

sensitisation profiles towards the 10 main major allergens greatly varies from north to south. The most prevalent IgE sensitisations are: vs Amb a 1 (15.6%) and Bet v 1 (25.8%) in north-western; vs Bet v 1 (17.3%) in north-eastern; vs Cup a 1 (27.9%) in center; vs Cup a 1 (21.3%), Ole e 1 (15.8%) and Par j 2 (20.7%) in southern; vs Ole e 1 (17.3%) and Par j 2 (20.4%) in islands. The distribution of IgE sensitisation vs Phl p 1-p 5, Der p 1, Der f 1, Der p 2, Der f 2, Can f 1, Fel d 1 e Alt a 1 is similar throughout the country.

Conclusion: Current epidemiology of allergic sensitisation is facing a new 'molecular era' which might lead to a detailed allergy 'geography'. Italy is characterised by a different distribution of IgE sensitisation profiles vs allergenic molecules, contributing to a global molecular vision of allergic sensitisation.

799

Simultaneous detection of total and allergen-specific immunoglobulin-E in human plasma using multiplex array technology

Lohman, MJ¹; Chapman, MD¹; King, EM¹
¹Indoor Biotechnologies, Inc., Charlottesville, VA, United States

Background: Methods used for quantitative measurement of immunoglobulin (Ig)-E in human serum or plasma such as ELISA or FEIA analyze one analyte per test, requiring a large sample volume for multiple analyses. Chip-based technology provides semi-quantitative results for fixed selection of multiple analytes. The aim of this study was to develop a flexible, quantitative multiplex array for total and specific IgE that simultaneously detects multiple analytes. The array was validated by com-

paring results with streptavidin-ImmunoCAP.

Method: IgE concentration in human plasma was detected using fluorescent microspheres covalently coupled to antibodies that were saturated with purified allergens. Human plasma samples ($n = 79$) were analyzed by 12-plex array and using purified allergen-specific streptavidin-ImmunoCAP to determine total IgE as well as IgE specific to Der p 1, Der f 1, Der p 2, Der f 2, Fel d 1, Can f 1, Mus m 1, Rat n 1, Bet v 1, Phl p 5, and Alt a 1. Performance criteria and reproducibility of the multiplex array as well as inter-method concordance were evaluated.

Results: IgE measurements by 12-plex array were reproducible both within and between assays: mean coefficients of variance (CV%) were 16.1% for intra-assay triplicates, and 12.6% for triplicate inter-assay analysis. The array demonstrated good parallelism between serial dilutions within the dynamic range (CV = 27.6%). Results obtained by multiplex array versus streptavidin-ImmunoCAP correlated closely (mean $r = 0.83$, $P < 0.001$), with a mean CV of 43.1%, and a concordance of 74.0%. The total sample volume required for the 12-plex test was 30 μ l compared to 700 μ l required for ImmunoCAP.

Conclusion: The multiplex array provides reproducible quantitative total and allergen-specific IgE measurements in human serum or plasma, while using only a fraction of the sample volume required in other quantitative methods. Our results suggest that fluorescent multiplex technology may facilitate future studies of allergic sensitisation. The multiplex platform can be adapted to include additional allergens and for measurement of IgE antibodies in animal models.

Poster Session 24

New data on the allergen biology

801

Highlighting potential targets of house dust mite allergenic proteases

Campisi, V^{1,2}; Herman, J¹; Bouaziz, A¹; Chevigné, A²; Galleni, M¹; Dumez, ME¹

¹Laboratory of Biological Macromolecules, Center for Protein Engineering, University of Liege, Liege, Belgium; ²Laboratory of Retrovirology, Public Research Center for Health, Strassen, Luxembourg

Background: The three allergenic proteases Der p 1 (a papain-like cysteine protease), Der p 3 and Der p 6 (serine proteases from the trypsin-like and the chymotrypsin-like family respectively) are secreted from the *Dermatophagoides pteronyssinus* house dust mite (HDM) specie. Their proteolytic activities represent adjuvant factors of the HDM-induced allergy pathogenesis, mainly through the cleavage of cell surface proteins which are directly involved in the outbreak and the chronicity of the allergic response.

Method: The interplay between a protease and its substrates is controlled in various ways, including the substrate recognition by the catalytic cleft. A good understanding of a protease specificity could be useful to identify its putative relevant human targets. We choose to characterise the extended substrate specificities of Der p 1, Der p 3 and Der p 6 using the 'substrate phage display' technology in order to predict *in silico* their targets that would play a potential role in allergy and inflammation.

Results: We engineered three phage display libraries in the fd-Tet-DOG phage, namely 'NNK', 'ARG' and 'TWT'. In these libraries, phage particles express random nonapeptides (positions P5-P4' totally randomised in the 'NNK' library) or partially random nonapeptides (position P1 restricted to a Lys or Arg residue in the 'ARG' library and to a Phe or Tyr residue in the 'TWT' library) which are in fusion with their surface exposed pIII protein. A simple screening protocol providing good enrichment rates was established. Three rounds of selection using the proteases were performed. It allowed us to highlight their different cleavage patterns and to build their specificity models. These models were then introduced into the Prediction of Protease Specificity (PoPS) bioinformatic tool in order to predict the potential sub-

strates of Der p 1, Der p 3 or Der p 6 in the human proteome.

Conclusion: Among these substrates, we have highlighted new potential targets which are mainly cellular receptors. We are now trying to classify them by phage ELISA in order to select the best substrates of the HDM proteases for further studies. This could help us to characterise the adjuvant role of allergenic proteases in the HDM-induced allergy pathogenesis.

802

New allergens involved in shrimp-mite cross reactivity

Gómez, C^{1,2}; Mazzeo, C^{1,2}; del Pozo, V^{1,2}; Boquete, M³; Sastre, J^{2,4}

¹Immuno-allergy, IIS-Fundación Jiménez Díaz, Madrid, Spain; ²CIBER de Enfermedades Respiratorias, Palma de Mallorca, Spain; ³Allergy, Hospital General de Calde, Lugo, Spain; ⁴Allergy, IIS-Fundación Jiménez Díaz, Madrid, Spain

Background: Tropomyosin is the major allergen described in seafood-arthropods cross-reactivity. Other allergens implicated in this cross-reactivity remain unknown. We sought to identify new allergens from *Solenocera melanthera* shrimp and which of them are involved in shrimp-mite cross-reactivity.

Method: Thirty-six patients from a dry city with continental climate (Madrid) and 37 patients from the coast (Lugo) were studied. The allergenic profiles to *S. melanthera*, and to *Dermatophagoides pteronyssinus* were analyzed by immunoblotting. The most prominent IgE binding proteins were analyzed by mass spectrometry. Also, inhibition assays were performed.

Results: The patients were classified in 2 groups: mite-seafood (MS) and seafood (S) allergic patients according to the clinical symptoms, skin prick tests and specific IgE. The patients IgE reactivity pattern (allergogram) to *S. melanthera* extract showed prominent bands that were analyzed by mass spectrometry. Tropomyosin, arginine kinase, sarcoplasmic calcium binding protein and new allergenic proteins like α -Actinin (~ 99 kDa), β -Actin (~ 47 kDa), Fructose biphosphate aldolase (~ 45 kDa) and Ubiquitin (~ 5 kDa) were identified.

When we compared *S. melanthera* to *D. pteronyssinus* allergograms, several differ-

ences between MS and S groups were found.

Interestingly, α -Actinin and Ubiquitin were recognised by both populations and in both extracts, suggesting that these proteins could be allergens implicated in mite-shrimp cross-reactivity. This data were confirmed by inhibition immunoblottings.

Conclusion: We have identified several new shrimp allergens: β -Actin, Fructose biphosphate aldolase, α -Actinin and Ubiquitin. Among these allergens, α -Actinin and Ubiquitin could be implicated in shrimp-mite cross-reactivity.

803

Reduction of the enzymatic activity in pollen native allergenic extracts after depigmentation and polymerization

Leonor, JR¹; Morales, M¹; Iraola, V¹; López-Matas, MA¹; Gallego, MT¹; Carnés, J¹

¹Laboratorios LETI, R & D, Tres Cantos, Spain

Background: The presence of different enzymes in allergenic extracts, including pollen, is important from two points of view. First the enzymatic activity plays a role in the pathogenesis of allergy; and the second point is the influence of these enzymes in the stability of the extracts. It has been demonstrated that Depigmented-polymerized (Dpg-Pol) extracts have a reduced enzymatic activity compared to their respective native extracts in mites, but there is little information regarding pollen. The objectives of this study were to characterise, and to compare the enzymatic activity of both type of extracts from different pollen extracts.

Method: Native and their corresponding Dpg-Pol extracts of pollen of five grass species (*Dactylis glomerata*, *Festuca elatior*, *Lolium perenne*, *Phleum pratense*, and *Poa pratensis*), one weed (*Salsola kali*) and one tree species (*Olea europaea*) were manufactured, and characterised by Lowry and SDS. The gelatinolytic activity was determined by Gelatin zymography in reducing and non-reducing conditions, the Cysteine protease activity by using the L-1460 peptide (Pyr-Phe-Leu-pNa) as substrate and papain as the standard, and 19 different enzymatic activities by the Api-Zym system.

Results: Gelatinolytic activity was detected in non-reducing conditions in native extracts from all species (except for *S. kali*) mainly around 45, 65 and above 100 kDa bands, but it was not detected in their corresponding Dpg-Pol extracts. Similarly, cysteine protease and most of the enzymatic activities detected by Api-Zym in native extracts from all species, were inactivated almost totally in the Dpg-Pol extracts.

Conclusion: In native pollen extracts from different species diverse enzymes are present, including proteases. The depigmentation-polymerization of the pollen extracts eliminates most of the enzymatic activity, suggesting a higher safety and stability in these chemically modified extracts.

804

Induction of Th2 cytokines by the cysteine protease allergen Der f 1 from murine basophils via G-protein coupled receptor dependent pathways

Yi,M-H¹; Jeong, KY²; Kim,HP¹; Kim, TY¹; Kim,CR¹; Yong,TS¹

¹Environmental Medical Biology, Institute of Tropical Medicine, Arthropods of Medical Importance Resource Bank, Yonsei University College of Medicine, Seoul, Korea; ²Department of Internal Medicine, Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea

Background: Der f 1, a major allergen of house dust mite, is a member of papain-like cysteine protease family whose proteolytic activity can provoke Th2 immune response. However, the innate immune recognition mechanisms and immune cells associated with the initiation of Th2 responses have not been investigated in detail. The objective of this study is to evaluate the effect of G-protein coupled receptors (GPCRs) on mouse bone marrow-derived basophils (BMBs) by the stimulation of cysteine protease allergen, Der f 1.

Method: Mouse bone marrow-derived basophils were stimulated with proteolytically active recombinant Der f 1 (rDer f 1) expressed in the yeast *Pichia pastoris*. Then the expressions of Th2 cytokines, IL-4 and IL-13 were examined by real time PCR and ELISA.

Results: Transcription of both IL-4 and IL-13 in mouse basophils was induced by the treatment of rDer f 1 with a cysteine protease activity assessed by real time PCR, whereas inactive rDer f 1 did not. Proteolytically active rDer f 1 induced production of IL-4 and IL-13 from basophils as measured by ELISA, while heat-treated rDer f 1 did not able to induce cytokine productions. However, Der f 1-induced IL-4 and 13 secretion in mouse basophils were

dramatically inhibited by the treatment of GPCR with *Pertussis* toxin.

Conclusion: These findings suggest that GPCRs are involved in secretion of TH2 cytokine such as IL-4 and IL-13 by cysteine protease activity of Der f 1.

805

A preliminary proposal for the clustering of olive pollens into model cultivars on the basis of their allergenic content

Marco, FM¹; Morales, S²; Castro, AJ²; Salmerón, C²; Rodríguez-García, MI²; Alché, JDD²

¹INMUNAL SAU, R&D, Alcalá de Henares, Spain;

²Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, Spanish National Research Council (CSIC), Granada, Spain

Background: Olive pollen (*Olea europaea* -OE-) is a major cause of respiratory allergy among Mediterranean countries and elsewhere in the world. Olive germplasm is exceptionally wide, with more than 250 cultivars only in Spain. Important allergenic differences have been recognised among pollen from different cultivars, involving most relevant allergens like Ole e 1. These differences may influence the clinical performance of OE extracts depending on the sensitivity of individual patients to specific allergens, hence cultivar origin should be taken into account in the manufacture of allergen extracts. As handling and characterisation of a large number of cultivar extracts is impracticable under industrial and clinical standards, the present work intends to define a limited number of cultivar models, characterised by distinctive pollen allergen profiles.

Method: The Ole e 1, Ole e 2, Ole e 5 and Ole e 9 content was analyzed using specific antibodies in OE extracts prepared from pollen of ten olive cultivars: 'Picual', 'Manzanilla', 'Arbequina', 'Blanqueta', 'Cornicabra', 'Verdial', 'Lechín', 'Hojiblanca', 'Lucio' and 'Loaime'. Allergen semi-quantization was performed by SDS-PAGE and immunoblotting densitometry (Quantity One, Bio-Rad Laboratories), normalizing data for each allergen against the cultivar with the higher concentration. Threshold percentages were defined in order to characterise individual levels of an allergen for each cultivar as low, average and high.

Results: Levels of Ole e 2 and Ole e 5 were not informative. Based on Ole e 1 and Ole e 9 expression cultivars could be grouped in three categories:

High Ole e 1; high Ole e 9: 'Hojiblanca', 'Loaime'

High Ole e 1; low Ole e 9: 'Picual', 'Manzanilla', 'Lucio', 'Cornicabra', 'Lechín', 'Blanqueta'

Low Ole e 1; low Ole e 9: 'Arbequina', 'Verdial'

Conclusion: In this study we have defined a strategy for grouping of OE cultivars on the basis of differences in relevant allergen expression. This could add specificity to the use of OE extracts in diagnosis and therapy. This preliminary proposal should be implemented by further analyzing additional olive pollen allergens and other relevant cultivars, and testing for the clinical correlates of these allergenic differences.

806

Enriched mannose glycosylation contributes to Act d 2 allergenicity

Gómez-Casado, C¹; Palacín, A¹; Tordesillas, L¹; Garrido, M¹; Blanca, N²; Blanco, C²; Cuesta-Herranz, J⁴; Sánchez-Monge, R¹; Díaz-Perales, A¹

¹Centre for Plant Biotechnology and Genomics (Technical University Madrid), Pozuelo de Alarcón, Madrid, Spain; ²Allergy Service, Hospital Infanta Leonor, Madrid, Spain; ³Allergy Service, University Hospital La Princesa, Madrid, Spain; ⁴IIS-Allergy Service, Jiménez Díaz Foundation, Madrid, Spain

Background: Allergens are responsible for the Th2 response in patients as part of complex mixtures of proteins, fatty acids and other molecules. Plant allergens have hitherto been included in several protein families that share no common biochemical features. Their physical, biochemical and immunological characteristics have been widely studied, but no definite conclusion has been reached about what makes a protein an allergen. N-glycosylation is characteristic of plant allergen sources but is not present in mammals.

Methods: In this work, we report the isolation of the protein fraction (by chemical deglycosylation) and the N-glycan fraction (by extended treatment with proteinase K) of the major kiwifruit allergen, the thaumatin-like protein (TLP) Act d 2 and characterise their allergenic role.

Results: The comparison of the allergenic activity *in vitro* (ELISA) and *ex vivo*

(basophil activation test), as well as the ability to activate cells from the immune system (as measured by T lymphocyte activation assays and monocyte derived dendritic cell maturation assays) have demonstrated the existence of IgE epitopes in the sugar moiety and their importance in recognition by antigen-presenting cells, inducing the maturation of these cells.

Conclusion: The sugar moiety of some plant allergens is also involved in inducing Th2 sensitisation, although the protein fraction is necessary to maintain it over time. These findings could influence the development of new strategies for allergy immunotherapy.

807

Immunological and proteomics study to identify allergens from date palm (*Phoenix sylvestris*) pollen

Saha, B¹; Pandey, N²; Bhattacharya, SG¹¹Division of Plant Biology, Bose Institute, Kolkata, India;²Allergy and Asthma, MP Birla Research Centre, Kolkata, West Bengal, Kolkata, India

Background: Allergy to pollen grains has been well document. Date palm (*Phoenix sylvestris*) trees are found in large quantities in and around Kolkata, India. Apart from its fruit being used as a food and delicacies its pollen grain is reported to be highly allergenic though not much has been researched on it. Allergy clinics in Kolkata receive a substantial number of patients who are tested to be severely allergic to this pollen. An immunoproteomics approach has been employed to identify IgE reactive proteins from this pollen.

Method: The pollen grains were isolated by passing flower through mesh of different sizes. A crude pollen protein extract in phosphate buffer is prepared which is further purified by salting out and G25 Column elution. IgE specific ELISA and western blotting with patient specific sera from 10 patients was done. Alongside total protein was profiled on a 2D gel electrophoresis using 3–10 as well as 4–7 PI range. Immunoblotting was performed to specifically detect IgE reactive protein spots. Mass spectrometry employing MALDI TOF/TOF was used to identify the allergenic spots. Biochemical techniques like glycoprotein staining and periodate modification was carried out. In silico allergenicity prediction was also done with the identified proteins.

Result: Clinical data of 10 patients selected for study showed high specific IgE titer, SPT of +2 and +3 with the pollen extract. Western blotting confirmed the presence of numerous allergenic bands differentially expressed with patient sera. Two bands of 62 Kd and 29 Kd was found to be dominant in all. 2D gel with coomassie staining showed an average of 85 and 154 protein spots in 3–10 and 4–7(PI) respectively. Western blotting proved presence of 8 (3–10) and 15(4–7) IgE reactive proteins with three overlapping spots. No reactive spots were present in control sera. Mass spectrometry identified the protein spots and was validated by *in silico* allergenicity prediction tools. One of the dominant proteins was identified to be pectin esterase. Four allergic spots were found to be glycoprotein in nature and their glyco moeity was found to be necessary for allergenicity.

Conclusion: *Phoenix sylvestris* is an important aeroallergen causing severe allergic rhinitis in a susceptible population in India. The 62 Kd and 29 Kd is the major

allergen. Mass spectrometry identified the 62 Kd allergen as pectin esterase. All the identified proteins are validated as potent allergens by predicting softwares.

808

Relevant allergens across species – similarities and differences

Swan, NJ¹; Hutchings, JW¹; Newland, BJ¹; Mwange, JD¹; Hewings, SJ¹; Bullimore, AD¹; Skinner, MA¹¹Allergy Therapeutics, R&D, Worthing, United Kingdom

Background: Allergens can be categorised into homologous groups, however the method of grouping can be performed in a number of different ways.

Methods: A selection of diagnostic products were investigated using SDS-PAGE, trypsin digestion and mass spectroscopy. The peptide fragments were then searched against well-established protein databases through MASCOT in order to confirm the presence of proteins and allergens. The proteins and allergens that were found within the products were then analysed to find similarities and differences between the selected products. The proteins and allergens were analysed through taxonomic grouping, function and where possible structure.

Results: All of the samples returned good matches to proteins or allergens within the database, with >20% sequence coverage and high peptide and protein scores according to MASCOT. It was possible to group proteins and allergens into various different categories; this resulted in some unexpected similarity between species and highlighted the differences between products and allergens. For products where there are no sequenced allergens, some of the proteins fell into categories with known allergens. Indicating that they shared a common property, depending on the number and type of shared properties an insight was provided for the characteristics of the protein and its potential for further investigation.

The categories allowed a different perspective for grouping allergens and show what properties are widespread and not so common between allergens of different species. This method of analysing and categorising has provided more characterisation information on the grouping of allergens and offers indirect data for allergens that may have not previously been extensively studied.

Conclusion: Through looking at grouping of allergens in a different way, new investigatory allergen targets have been found. Re-grouping allergens according to a variety of different properties has led to a greater understanding of the characteristics of allergenic proteins.

809

Broad-spectrum immunotherapy – heterogeneity of biological profile of grasses within the family of sweet grasses and improved patient relevance of grass mix products

Bullimore, AD¹; Batten, TN¹; Skinner, MA¹¹Allergy Therapeutics, R&D, Worthing, United Kingdom

Background: Biochemical profiles of multiple grass species commonly found across Europe have been assessed to identify the advantages of multiple grasses in broad spectrum grass immunotherapy. The benefits for inclusion of multiple grasses in immunotherapy and potential limitations of Timothy grass exemplar therapy have also been identified by selectively blocking specific grass reactivities within patient serum.

Methods: A number of biochemical methods have been used to assess key parameters relating to similarities and differences in allergens from multiple grass species. Each grass was analysed for major allergen, IgG and IgE reactivity by inhibition ELISA and total protein content. Using sera from grass sensitive patients we have selectively blocked reactivity to specific grass allergens. This assay platform allowed us to distinguish whether there were any allergens in other grasses which were not cross-reactive with timothy grass exemplar allergens.

Results: Each of the grass species investigated is distinct in having a unique allergen fingerprint and biochemical profile as determined by radar graph. This suggests therapy composed of a mix of grasses potentially contributes protection to a more diverse grass sensitive population. Selective blocking of specific grass reactivities within patient serum showed that the majority of patients demonstrated reactivity to multiple grass allergens which were not present in timothy grass exemplar allergen extracts.

Conclusion: The biochemical and serological attributes of specific grass allergen reactivity support inclusion of multiple grasses for use in broad spectrum grass allergy immunotherapy.

810

Growth characteristics and major allergen (Blo t 5) content of custom manufactured and commercially available *Blomia tropicalis* mite cultures

Ardusso, L¹; Maspero, J²; Bullimore, AD³; Skinner, MA³

¹Allergy and Immunology Department, Rosario School of Medicine, Rosario, Argentina; ²Fundación CIDEA, Buenos Aires, Argentina; ³Allergy Therapeutics, R&D, Worthing, United Kingdom

Background: *Blomia tropicalis* (Bt) is highly prevalent in Latin America and therefore incidences of allergy are high. However, obtaining sufficiently potent allergen extracts can be difficult due to the potency and major allergen content being influenced by culture techniques and growth media. It is common that yeast is thought to be necessary in order to obtain highly populated cultures, however it is undesirable to include yeast grown mites in an immunotherapy. In this work we describe three different cultures grown with different media and the results obtained from the characterisation of these cultures and the resulting extracts in terms of the content of major allergen Blo t 5.

Methods: Two cultures were manufactured simultaneously; one grown on fish food with added yeast and the other with desiccated Porcine liver with no yeast. Once the crop reached the final phase of development, the culture was frozen at -20°C to kill the mites. Thereafter the culture was passed through graded sieves with different pore sizes.

The mites were separated from faecal matter and remaining culture media and desiccated for 2 weeks. These two cultures were compared against a commercially available *B. tropicalis* culture for Blo t 5 content via identically produced extractions.

Results: Blo t 5 content varied across culture type. The commercially available extract contained less than half the Blo t 5 of the culture grown on Fish food with yeast supplements and more than four-times less than the culture grown on desiccated Porcine liver (26307.3 ng, 70705.5 ng and 148885.7 ng respectively).

Conclusions: It is possible to grow *B. tropicalis* cultures without yeast supplementation on desiccated Porcine liver and achieve higher concentrations of Blo t 5 than when grown on yeast supplemented media.

811

Determination of HDM group 1 and 2 isoforms using different antibody based assays

Österberg, M¹; Skydtsgaard, MB²; Christensen, LH²; Hansen, GN²; Larsen, G²; Andersen, LB²; Bergmann, K²; Lund, L²; Henmar, H²

¹ALK-Abelló, Hørsholm, Denmark; ²CMC Research, ALK-Abelló, Hørsholm, Denmark

Background: Major allergens are often quantified by assays using antibodies, however as allergens often exists as different isoforms, parts of these allergens may not be quantified, as the antibodies may not bind to all isoforms. In these experiments we have investigated several antibodies capability of binding to different isoforms of group 1 and 2, in *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* extract.

Method: Mite extract were subjected to Iso Electric Focusing (IEF) by using an Offgel Fractionator to separate the isoforms. The instrument only separate with regard to pH, therefore we can only conclude on mutations which do have an effect on the pH. These fractions were analysed in rocket immuno electrophoresis using both poly and monospecific polyclonal antibodies (pAbs), and in specific ELISA assay and Biacore using monoclonal antibodies (mAbs). Finally the isoform variation of group 1 and 2 in the fractions were explored by Mass spectrometry (MS).

Results: From the IEF of the two extracts, we were able to define the fractions where either group 1 or 2 were present using a polyclonal antibody raised towards the whole extract. This result was compared with the specific allergen ELISA results and Biacore data using mAbs. From these data we can conclude, that some mAbs can bind to a similar spectre of isoforms as the pAb. Further similar ratio between the different isoforms was detected with both pAbs and mAbs. MS analysis identified the fractions containing group 1 or 2 and this correlated well with the findings in the antibody based assays.

Conclusion: We were able to identify, that the monoclonal antibody tested could identify the same range of isoforms as the polyclonal antibody. Further the pAb and mAbs assays correlated well with regard to level of these isoforms.

812

Immunological characterisation of the bacterial Bet v 1 homologues from *Thermus thermophilus* and *Streptococcus mutans*

Ackerbauer, D¹; Smole, U²; Gepp, B¹; Breiteneder, H¹

¹Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria; ²Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Background: The major birch pollen allergen Bet v 1 is a member of the pathogenesis related protein family 10. The structures of Bet v 1 and the bacterial homologues from *Thermus thermophilus*, a gram-negative thermophilic bacterium, and *Streptococcus mutans* are closely related although they possess only low sequence similarity. *S. mutans* is a gram-positive oral bacterial pathogen whereas exposition to *T. thermophilus* is highly unlikely. The aim of this study was to produce and characterise the Bet v 1-related proteins SMU.440 (PDB 3IJT) of *S. mutans* and TTHA0849 (PDB 2D4R) of *T. thermophilus* and to elucidate a possible link between antibody responses to Bet v 1 and its bacterial homologues.

Method: The proteins SMU.440 and TTHA0849 were expressed in *E. coli* BL21 (DE3) cells. After cell lysis by French Pressure Cell Press and ammonium sulfate precipitation, the proteins were purified by consecutive hydrophobic interaction, anion exchange and gel filtration chromatography. The secondary structures of the proteins were evaluated by circular dichroism (CD) spectroscopy. The purified proteins were tested for their IgE, IgG, IgG4 and IgA reactivity in direct ELISA using the sera of birch pollen, house dust mite and grass pollen allergic patients as well as normal human sera (NHS).

Results: The purified SMU.440 and TTHA0849 were detected as single 16 kDa and 17 kDa protein bands in SDS-PAGE, respectively. The bacterial homologues showed secondary structures in CD spectroscopy similar to recombinant Bet v 1. Neither SMU.440 nor TTHA0849 showed IgE reactivity with sera of 16 birch pollen allergic patients. Interestingly, IgG and IgG4 specific for SMU.440 and TTHA0849 could be detected in sera of birch pollen, house dust mite and grass pollen allergic patients as well as in NHS. SMU.440 also showed IgA binding.

Conclusion: In this work, supported by grant SFB-F4608 from the Austrian Science Fund, we obtained first insights into the immunological properties of the bacterial Bet v 1 homologues SMU.440 and TTHA0849.

813

Purification and characterisation of the major allergens from house dust mite

Österberg, M¹; Friberg, L²; Hansen, GN²; Amondson, P²; Gisselsson, A²; Christensen, LH²; Henmar, H²
¹ALK-Abelló, Hørsholm, Denmark; ²ALK-Abelló, CMC Research, Hørsholm, Denmark

Background: Inhalation allergy to house dust mite is among the most prevalent respiratory allergies worldwide. More than 20 allergen molecules have so far been identified and characterised from house dust mites. We have by conventional methods purified the major allergen, group 1 and 2, from *Dermatophagoides pteronyssinus* (Der p) and *farinae* (Der f) and characterised these allergens with respect to purity, folding, isoform variation and mass spectrometry (MS) analysis.

Method: Acetone precipitated mite culture were used as starting material for the purification of the allergens. By a combination of several different columns such as hydrophobic interaction, affinity (chelate) and size exclusion we were able to purify the major allergens. The purified allergens were characterised by Crossed immuno electrophoresis (CIE), SDS-PAGE, Isoelectric focusing (IEF), Circular Dichroism (CD) and MS.

Results: The major allergens (mix of isoforms) were visualised as one dominant band in a SDS-PAGE and 1–2 more faint bands. CIE showed only one distinct precipitate. All bands from SDS-PAGE were identified as the respective major allergen

in MS analysis. CD analysis showed that Der p 1 and Der f 1 had predominantly α -helix structure, whereas group 2 allergens showed a β -sheet structure. IEF of the major allergens showed that group 1 isoforms are found from pI 4.9 to 7.3 and group 2 isoforms from pI 5.9 to 8.3. Further we found that the spectre of isoforms were similar to the isoforms found in the starting material.

Conclusion: It is possible from a mite extract to purify the group 1 and 2 allergens using conventional methods. The purified allergens were identified by MS. The major allergens from Der p and Der f contained several isoforms, which could be group by MS. It is important in quantification assay to develop assays which can quantify all isoforms.

814

German cockroach extract modulates MMP-9 and tight junction integrity in human airway epithelial cells

Lee, KE¹; Hong, JY¹; Kim, MN¹; Heo, WI¹; Kim, KW¹; Kim, KE¹; Sohn, MH¹
¹Institute of Allergy, Biomedical Science Institute, Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Pediatrics, Seoul, Korea

Background: Exposure and sensitisation to cockroach allergens are associated with the development of acute asthma morbidity. Matrix metalloproteases (MMPs) have been suggested to play an important role in remodeling of the airways in disease.

However, the mechanism by which cockroach-driven MMP activity leads to asthma pathogenesis and remodeling remains unclear. Thus, we investigated whether German cockroach extract can directly trigger MMP-9 and the MMP-9 activation affects the change of tight junction protein integrity.

Method: In this study, MMP-9 protein was measured by ELISA against human MMP-9. Specific antibodies to tight junction proteins were used for Western blotting. Pharmacological inhibitors for protease from German cockroach and MMP activity were incubated with cells before German cockroach treatment.

Results: In human airway epithelial cells, German cockroach extract increased MMP-9 mRNA and protein depending on time and dose. However, tissue inhibitor of metalloprotease (TIMP1) expression was not significantly detected. Moreover, twenty four hours after German cockroach extract treatment, tight junction proteins such as ZO-1 and Occludin gradually decreased on Western blotting analysis. Pretreatment with either serine protease inhibitor or Ro32-3555, a specific MMP inhibitor significantly prevented the MMP-9 protein production and the tight junction protein decrease.

Conclusion: Taken together, these data suggest that German cockroach extract directly induces MMP-9 in human airway epithelial cells and may contribute to disruption of epithelial barrier function.

Poster Session 25

Management of food allergy I

815

Evaluating the potential risk of allergy from a new pest resistant genetically modified soybean line

Lu, M¹; Kong, XX²; Ladics, GS³; Goodman, RE¹

¹Food Allergy Research and Resource Program, University of Nebraska-Lincoln, Lincoln, NE, United States; ²Statistics, Pioneer Hi-Bred International, Johnston, IA, United States; ³DuPont Experimental Station, Regulatory Sciences, Wilmington, DE, United States

Background: The Codex Alimentarius Guideline (2003) for food safety assessment of GM plants recommends evaluating potential changes in endogenous allergen expression if the plant is a common source of food allergy. The European Food Safety Authority (EFSA) now asks for direct enzyme-linked immunosorbent assay (ELISA) and both 1D and 2D immunoblotting using individual allergic sera for IgE binding comparisons to evaluate diverse epitope recognition. Because the natural variability in the expression of endogenous allergens between varieties is unknown, a new pest-resistant genetically modified (GM) soybean, a near-isogenic line and five non-GM commercial lines of soybeans grown in multiple geographical regions were compared for IgE binding.

Method: Qualitative differences were evaluated from IgE binding to proteins separated under reducing and non-reducing 1D-SDS-PAGE and 2D-IEF x SDS-PAGE. Quantitative differences were compared by direct ELISA. Eleven individual soybean allergic sera and five non-soybean allergic control sera were tested.

Results: Minor IgE binding differences were observed between the GM and near-isogenic soybeans as well as some commercial lines for some subjects by immunoblotting. The IgE binding measured by ELISA was not statistically different between the GM and near-isoline control soybeans and was within the range of variation of the other commercial soybeans lines with different genetic backgrounds.

Conclusion: The evidence suggests that this GM soybean line does not present an increased risk of allergy as the variation in IgE binding was similar to commercial soybean lines. More importantly, soybean allergic consumers should avoid consumption of foods containing any soybeans,

thus the relevance of the required testing is not clear.

816

Comparison of two extensively hydrolyzed formulas with probiotics for the treatment of cow's milk protein allergy

Vandenplas, Y¹; Steenhout, P^{2,3}; Planoudis, Y³; Grathwohl, D^{2,3}; Hauser, B⁴; Althera Study Group (J. Christens, C. Halut, B. Hauser, T. Devreker, S. Mullier, P. Mariën, G. Veereman, K. Kamoën, S. Peeters, F. Smets, F. Bury, M. Verghote, P. Bollen, O. Beauraind, P. Lenoir, S. Colinet, M. Van Winckel)

¹UZ Brussel, Pediatrics, Brussels, Belgium; ²Nestle Nutrition, Vevey, Switzerland; ³Nestle Clinical Development Unit, Lausanne, Switzerland; ⁴Althera Study Group, Brussels, Belgium

Background: According to guidelines, the recommended treatment of cow's milk protein allergy (CMPA) is a strict elimination diet with an extensive (cow's milk based) hydrolysate. Whether the best option is a whey or a casein hydrolysate is a matter of debate.

Methods: We compared the efficacy of an extensive whey to that of an extensive casein hydrolysate both enriched with (different) probiotic strains, in a double-blind, randomised, trial. Efficacy was assessed with a novel clinical score. The score encompasses crying, regurgitation, stool consistency, atopic eczema, urticaria and respiratory symptoms. The score ranges from 0 to 33. The primary hypothesis of the trial was to show non-inferiority between the whey and casein hydrolysate in means of the score. The inclusion criterion of the trial was a clinical suspicion of CMPA because of a score ≥ 12 . Specific and total IgE and skin prick test (SPT) were performed at baseline. The composition of the gastrointestinal flora was analysed at baseline and after one month.

Results: One hundred and sixteen infants with a clinical score of ≥ 12 were included. There was a statistical and clinical significant decrease of the score during the first month: -8.32 in the whey and -7.82 in the casein hydrolysate group. The treatment difference at one month was -0.879 (95% CI $-2.79, 1.03$), confirming that the whey hydrolysate is non-inferior to the casein hydrolysate. An open challenge was performed in 85 (74%) infants and was

positive in 59/85 (69%); there were less late reactions in the whey (8/41 (20%)) than in the casein hydrolysate group (18/44 (41%), $P = 0.037$). No significant differences were found for total and specific IgE, and for the SPTs. No significant differences were found in the score-composing items: crying, regurgitation, stool consistency, atopic eczema, urticaria, respiratory symptoms. The whey hydrolysate, which was enriched with bifidobacteria, did lead to more bifidobacteria, enterobacteria and total bacteria but to less lactobacilli. The casein hydrolysate, which was enriched with lactobacilli, resulted in higher lactobacilli levels. The whey hydrolysate leads to better growth (weight and weight for age z-scores); the casein hydrolysate leads to smaller head circumferences.

Conclusion: In daily practice, the clinical score of ≥ 12 is a useful tool to select infants with a high risk of IgE and non-IgE mediated CMPA. The extensive whey and casein hydrolysates are equally effective in the treatment of CMPA.

817

Clinical reactivity to baked milk (cake) and products with pasteurised milk (yoghurt, feta cheese) may determine different phenotypes of cow's milk allergy

Arik Yilmaz, E¹; Cavkaytar, O¹; Buyuktiyaki, B¹; Soyer, OU¹; Tuncer, A¹; Sackesen, C¹

¹Department of Pediatric Allergy, Hacettepe University Faculty of Medicine, Ankara, Turkey

Background: Prior studies determined that thermal processing largely destroyed IgE binding epitopes in milk allergic children. Recently few studies have shown that approximately 80% of children with milk allergy were tolerant to baked milk. According to our observations, some children who were reacted to direct milk were tolerant to baked or pasteurised milk products like cake, yoghurt and feta cheese. Our aim is to identify characteristics of cow's milk allergic children who are tolerant to baked or pasteurised milk products.

Method: Children with IgE-mediated cow's milk allergy who had clinical reaction during DBPCFC tests with milk or positive history of anaphylaxis with milk in last

6 months were included in this study. Open food challenge tests were performed with baked milk (a piece of cake containing 1 gr milk protein) and products of pasteurised milk (a cup of yoghurt containing 0.9 gr milk protein and feta cheese containing 1.5 gr milk protein).

Results: Thirty-three children (4.1 ± 0.4 years, mean \pm SEM) with positive DBPCFC or history of anaphylaxis in the last 6 months underwent open food challenge tests with cake, yoghurt and feta cheese, respectively. Eighteen children (54.5%) were tolerant to baked milk (cake) and products of pasteurised milk (yoghurt or feta). Fifteen children had clinical reaction; 4 during baked milk challenge (cake), 11 during products of pasteurised milk challenge (yoghurt, feta). Initial cow's milk sIgE levels were 29.8 ± 7.2 kU/l in tolerant group; 40.7 ± 14.7 in reactive group with yoghurt/feta and 67.4 ± 21.6 in reactive group with cake. Similarly total IgE levels were 245.7 ± 87.1 kU/l, 266.6 ± 75.2 and 425.5 ± 155 in tolerant children and reactive children with yoghurt/feta and reactive with cake, respectively. Skin prick test (SPT) reactivity with direct milk was 8.6 ± 0.8 mm, 7.4 ± 0.8 and 14.8 ± 4.7 in groups, respectively. But SPT reactivity with milk allergen was similar in three groups. Cow's milk sIgE at open food challenge tests were 22.1 ± 6.7 kU/l; 27.3 ± 9.9 and 75.9 ± 30.1 in groups, respectively. The amount of milk that was tolerated at DBPCFC test was 48.4 ± 11.7 ml, 11.2 ± 3.1 ml and 6.4 ± 4.3 ml in groups, respectively.

Conclusion: Our results show that skin prick test reactivity with milk but not milk allergen, the amount of milk that can be tolerated and initial, follow-up milk sIgE measurements may define children who are reactive to baked milk (cake) or products containing pasteurised milk (yoghurt and feta cheese).

818

Development of a measure to determine the impact of food allergies on the daily lives of food allergic adults

Peniamina, R¹; Miroso, M¹; Bremer, P¹; Conner, T²

¹Department of Food Science, University of Otago, Dunedin, New Zealand; ²Department of Psychology, University of Otago, Dunedin, New Zealand

Background: Research on the quality of life of food allergic adults generally involves the use of health-related quality of life questionnaires (both generic and food allergy specific). A limitation of this approach is that it requires participants to accurately recall and report on past experi-

ences. The reality of autobiographical memory is that even relatively important events in people's lives can be forgotten over time and frequent behaviours are unlikely to be remembered on an individual basis. The aim of the current study was to develop a quantitative real-time data capture method that enables participants to report on their current behaviours and experiences, thus removing the bias introduced by autobiographical memory.

Method: Four focus groups were held with food allergic adults (total $n = 29$, age 20–77, variety of different food allergies) to obtain an in-depth understanding of the food allergy-related issues impacting on their quality of life. Thematic analysis of the focus group transcripts was completed using the NVIVO 9 software package. A short questionnaire was developed based on the focus group results.

Results: The resulting questionnaire was designed to be administered online on a daily basis for a given period of time (e.g. across two weeks). The 24 food allergy-related items (grouped within seven categories: allergen-free eating, financial cost, time cost, personal cost, external influences, physical effects, psychological issues) were derived from the themes identified from the focus groups data. Participants can select which items impacted their lives on that day. The three items with the most impact that day can then be rated (in terms of perceived stress imparted and impact on ability to participate in normal daily tasks). Additional items assess perceived mood and stress levels for the day.

Conclusion: This new measure can accurately provide information on the frequency and level of impact (stress imparted and impact on ability to participate in normal daily activities) of different issues in the daily lives of food allergic adults. Such food allergy knowledge may lead to the development of interventions that will benefit the daily experience and well-being of food allergic individuals. In combination with demographic and food allergy information it will also be possible to learn more about different sub-groups within the food allergic population.

819

Quantitative evaluation of antigenicity of cooked egg and egg products at antigen component level for the use of dietary counseling

Ito, S¹

¹Food Science and Nutrition, Doshisha Women's College of Liberal Arts, Kyoto, Japan

Background: The diagnosis of offending antigen in food allergy is going to be

pursued adequately according to Japanese Pediatric Guideline for Food Allergy 2012 in Japan. Hen's egg is the most prominent allergen in Japanese infants with both infantile atopic dermatitis and anaphylaxis. Ovalbumin (OVA) and ovomucoid (OM) are known as major allergens of hen's egg. In our clinical data about 1793 infants with positive egg white specific IgE antibody, which is equivalent to OVA specific IgE antibody, only 66.3% of them showed positive OM specific IgE antibody. Moreover, antigenicity of OVA and OM change independently for each other by cooking. To pursue dietary counseling safely and effectively for the purpose of early induction of tolerance, quantitative evaluation of egg antigen in cooked egg and egg products at antigen component level is required. The purpose of this study is to make a list of egg allergen exchange based on quantitative evaluation of OVA and OM in cooked egg and egg products. The list is to be made for patients with positive and negative OM specific IgE antibody respectively.

Method: Quantitative evaluation of OVA and OM in cooked egg and egg products was made using sandwich ELISA system developed for the detection of food antigen in processed food. By modification of this system, quantitative evaluation of OVA and OM in cooked egg and egg products was made.

Results: The discrepancy of antigenicity between OVA and OM was biggest in boiled eggs. The total amount of OVA in a hard-boiled egg of 50 g was 1.2 mg and that of OM was 1000 mg. This data imply that most of egg allergic patients with no OM specific IgE antibody in sera can tolerate one hard-boiled egg, but may not tolerate even one bolo which contain 2–8 mg of OVA and OM respectively in a bolo of 0.5–1 g. The antigenicity of egg and egg products can be classified into three groups. Group 1 (OVA:OM = 1:1,000): OM predominant group such as a hard-boiled egg, Chawan-mushi (A custard-like egg and vegetable dish steamed in a cup), Group 2: (OVA:OM = 1:1): OVA and OM express their antigenicity equally such as scrambled egg, cookies, bolos, and Group 3: (OVA>OMA): OVA predominant group such as baumkuhen, sponge cake, chiffon cake. Lists of OVA and OM exchange was made from these data.

Conclusion: Lists of egg allergen exchange based on the major allergen OVA and OM was made. These lists will make it possible to apply the result of food challenge test to dietary counseling aiming at outgrow.

821

Review of baked egg challenges at a tertiary pediatric allergy centre

Turner, PJ^{1,2}; Mehr, S³; Joshi, P³; Tan, J³; Wong, M³; Kakakios, A^{2,3}; Campbell, DE^{2,3}

¹Paediatric Allergy, Imperial College London, London, United Kingdom; ²Paediatric Allergy & Immunology, University of Sydney, Sydney, Australia; ³Paediatric Allergy & Immunology, Children's Hospital at Westmead, Sydney, Australia

Background: Many children with IgE-mediated allergy to egg can tolerate egg in baked foods. This removes the need for strict avoidance of egg in the diet and may significantly improve quality of life. The clinical characteristics and severity of reactions of egg allergic children who react to baked egg at oral food challenge (OFC) are not well defined.

Methods: All children presenting to our tertiary referral clinic with a diagnosis of egg allergy (on the basis of convincing clinical reaction in past 12 months and/or serum specific IgE or skin prick test (SPT) to egg >95% PPV) were offered OFC to baked egg. Challenges were performed with incremental dosages to a total of one baked muffin containing 1/6 egg (equivalent to 1 g egg protein) following a standardised protocol and recipe. Data was collected prospectively from 2009–2012.

Results: OFC were carried out on 240 children who were strictly avoiding egg in their diet and met the above inclusion criteria. 31 (13%) had experienced previous anaphylaxis to egg. 87 children (36%) reacted to their challenge. 13 children (15%) reacted to 60 mg protein or less, 35 (39%) to less than 250 mg (equivalent to ¼ muffin). 71% of children with prior anaphylaxis to egg passed OFC to baked egg. Of those children who reacted, 14% experienced anaphylaxis to baked egg, according to WAO criteria (5% of total cohort). Four of these children reacted to less than 100 mg protein. Intramuscular adrenaline was administered to five children, one of whom required a second dose due to persistent hypotension.

The most common reported reactions were: urticaria (37%), itchy mouth and/or throat (31%), abdominal pain (28%) and vomiting (20%). 94% of reactions occurred within 1 h of the initiation of the challenge, the remainder were gastrointestinal symptoms.

The size of the SPT to egg white in children with positive reaction on muffin challenge (mean 9.0 mm, SD 3.7) was not significantly different from those with negative challenges (8.7 mm, SD 3.1). Children with positive OFC were more likely to have had anaphylaxis to other foods (21% vs 10%). Multiple food allergies or

asthma (on preventer therapy) was not predictive of challenge outcome.

Conclusion: The majority of children with IgE-mediated allergy to egg are able to tolerate small amounts of baked egg. A proportion of children in this cohort reacted with symptoms of anaphylaxis. We therefore recommend that OFC to baked egg should take place under medical supervision.

822

Parental knowledge of anaphylaxis treatment and epinephrine autoinjector for children with food allergy

Contreras-Porta, J¹; Ruiz-Baqués, A²; Capel, F³; Ariño, M⁴; Zorrozua, A⁵; López-Serrano, M⁶

¹Hospital Universitario La Paz, Servicio de Alergia, Madrid, Spain; ²Ideas en Salud, Barcelona, Spain; ³AEPNAA, Murcia, Spain; ⁴Inmunitas Vera, Barcelona, Spain; ⁵Elikalte, Bilbao, Spain; ⁶Hospital Universitario La Paz, Madrid, Spain

Background: The purpose of this study was to gain a clearer understanding of Spanish parents' knowledge on how to treat anaphylactic reactions and epinephrine autoinjector (EA).

Method: An online anonymous-questionnaire format was used to collect details on treatment of food anaphylaxis and knowledge of epinephrine autoinjector (EA). The 25 items survey had been developed under patient-centered care, self-management and the participatory medicine model, agreed upon by the Spanish patient associations (AEPNAA, Inmunitas Vera and Elikalte) and by health professionals and researchers. 215 parents of food-allergic children between the ages of 1 and 15 (87 male and 128 female) answered an ad-hoc questionnaire.

Results: The majority (72%) had had one or more anaphylactic reactions and only 26% had received treatment with epinephrine. Almost all of parents knew EA (97%), were not prescribed in emergency departments nor primary health offices, but mainly prescribed by allergy specialists (74%). 15% carry EA sometimes, 64% always and 21% almost always. Only 25% of the families never received a demonstration of the use of EA, but 45% received demonstration by the physician of allergy office and 34% by a patient association or both, not in emergency departments. 70% of parents felt that they could know when to use an EA in an emergency. Only 24% of parents considered nurses and doctors as the main source of information on food allergy, and below 40% patients' associations and 32% internet sources respectively.

Conclusion: Epinephrine Auto-injector is under-used in parents of food allergic

children. Anaphylaxis treatments are often poorly known, and patients' associations are considered as the main source of information on food allergy.

823

Multicenter validation of a mouse model for cow's milk allergy to assess the allergenicity of hydrolysed cow's milk based infant formulas; phase III

van Esch, BCAM¹; van Bilsen, J²; van Gros-Hest, M³; Jeurink, PV⁴; Garssen, J^{1,4}; Smit, JJ⁵; Pieters, RHH⁵; Knippels, LMJ⁴

¹Immunopharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands; ²TNO, Risk Analysis for Products in Development, Zeist, The Netherlands; ³FrieslandCampina DOMO, Ingredients Innovation, Wageningen, The Netherlands; ⁴Danone Research Centre for Specialised Nutrition, Wageningen, The Netherlands; ⁵Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

Background: This study is part of a multi-phase project which aims to validate a mouse model to assess the potential allergenicity of hydrolyzed cow's milk based infant formulas (claim support EC-directive 2006/141/E). Previous results showed its transferability and its ability to discriminate between the sensitising properties of whey protein and extensively hydrolysed whey protein (eWH). In this phase the sensitising properties of three partially hydrolyzed whey proteins (pWH-A,-B,-C) were included.

Method: Mice received intragastric administration of PBS, whey, pWH or eWH at weekly intervals for 5 weeks using cholera toxin as an adjuvant. One week after the last sensitisation, anaphylactic shock symptoms, body temperature and an acute allergic skin response were determined as allergic parameters upon intradermal ear injection of whey. Subsequently, mice were challenged intragastrically with whey after which serum was analyzed for whey-specific antibodies and mMCP-1 as a reflection of mast cell degranulation. All protocols, test substances and procedures were standardised.

Results: Consistent with phase I and II, elevated levels of whey-specific IgE/IgG1 were detected in whey sensitised mice in all participating centers. In the current study, three out of four centers showed allergic symptoms in the positive control upon whey challenge measured as anaphylactic shock symptoms, a drop in body temperature, serum mMCP-1 or acute allergic skin responses. No allergic symptoms were observed in pWH-A sensitised mice although levels of whey-IgE were measured in a few animals. Individual animals sensitised with pWH-B and pWH-C showed minor clinical symptoms in addition to

wey-IgE, although the group mean values of mMCP-1, anaphylactic shock symptoms, body temperature and acute allergic skin response did not differ from non-sensitised mice. In contrast, none of the evaluated parameters were altered in eWH-sensitised mice.

Conclusion: In contrast to eWH, individual animals sensitised with pWH showed minor allergic symptoms. Measurement of clinical symptoms in addition to IgE is indispensable to predict allergenicity of hydrolysed whey proteins. Centers were able to discriminate between the sensitising properties of whey protein, pWH and eWH. The results demonstrate the discriminatory power of the mouse model and thereby the potential to evaluate the allergenicity of hydrolysed cow's milk formulas although the value of the different parameters measured need to be evaluated.

824

Removal of peanut allergen from different surfaces with household and hospital cleaning wipes

Watson, WT¹; Woodrow, AM²

¹Pediatrics, Dalhousie University/IWK Health Centre, Halifax, NS, Canada; ²IWK Health Centre, Halifax, NS, Canada

Background: A diagnosis of peanut allergy in children cause caregivers major concern as exposure to even small amounts could trigger an allergic reaction. Accidental contamination of toys and other products in areas where individuals have eaten may occur in places such as hospital cafeterias and waiting rooms. We explored whether cleaning peanut contaminated products with a common household and hospital cleaning wipes would remove peanut allergen.

Method: A nonporous table surface, plastic doll, textured plastic ball, book cover with a smooth surface, book cover with a textured surface, and a standard hospital tray were sampled for peanut allergen Ara h 1 at baseline. All items were smeared with peanut butter in a standard fashion. After drying for 5 min all products except the hospital tray were cleaned with a common household wipe (Clorox® disinfecting wipes, active ingredients n-alkyl dimethylbenzyl ammonium chloride 0.145%, n-alkyl dimethyl ethylbenzyl ammonium chloride 0.145%, isopropanol 1–5%), or two hospital cleaning wipes (Ultrawipes™, active ingredient didecyl dimethyl ammonium chloride 0.1%; PerCept RTU Wipes™, active ingredient hydrogen peroxide 0.5%). The hospital tray was washed within 24 h by a standard hospital automatic dish-

washer. After cleaning all products were sampled for Ara h 1.

Results: At baseline, no Ara h 1 was found on any product. Detectable Ara h 1 was found on all products after applying peanut butter. All wipes successfully removed all detectable Ara h 1 from all products. Standard washing of the hospital tray also removed all detectable Ara h 1.

Conclusion: Table surfaces, book covers and plastic toys can be successfully cleaned to remove peanut allergen using common household or hospital cleaning wipes. This should help reduce caregivers concerns in places where books and toys may be used by many children. Regular cleaning of these products or cleaning prior to use should be promoted to reduce risk of accidental peanut exposure.

825

Modern approaches to the treatment of cow's milk protein allergy in infants of first year of life

Namazova-Baranova, L^{1,2}; Vishneva, E¹; Alekseeva, A^{1,2}; Levina, J^{1,2}; Borovik, T^{1,2}; Makarova, S^{1,2}
¹Scientific Center of Children Health, RAMS, Moscow, Russia; ²First Moscow State Medical University named by IM Sechenov, Moscow, Russia

Background: Prevalence of food allergy, especially to cow's milk protein, amongst infants of first year of life increases everywhere. Hypoallergenic formulas, which do not cause allergy (in 90% of children, according to ESPGHAN&ESPACI) have been recommended for noninvasive diagnostics and treatment.

The objective of the study was an evaluation of effectiveness and safety of using of the amino-acid based formula in the real clinical practice.

Method: An open prospective study has been conducted in 2010. There were 60 children participating ranging in age 1–11 month with various degree of cow's milk protein allergy. Amongst them 52% had cutaneous and gastrointestinal (GI) allergy signs, 38% had respiratory signs in addition to it, 5% had cutaneous and respiratory signs and 5% had only cutaneous signs. 90% of children had GI signs of cow's milk protein allergy of various intensity.

Results: The conducted analysis of patients diet showed that prior to visiting the Center's pediatrician or neonatologist, 43% of the children received soy formula, 13% goat milk based formula, 12% partially hydrolyzed formula and 32% extensively hydrolyzed formula.

All children were prescribed an amino-acid based formula for 14–28 days. Before the beginning of the study and after 14–

28 days of therapy, including being on amino-acid based formula, the extent of symptoms of cow's milk protein allergy was evaluated using a specially designed questionnaire. The intensity of symptoms before and after administration of the amino-acid based formula was: GI signs 5 ± 1.2 and 0.6 ± 0.08 points (accordingly, $P < 0.001$), skin signs -3.9 ± 1.15 and 0.5 ± 0.08 p. (accordingly, $P < 0.01$), respiratory signs -0.7 ± 1.12 and 0 ± 0.08 p. (accordingly, $P < 0.01$). Negative side effects were noted in five children (8%): restlessness and nap disturbance in 1 child (unable to find a cause-and-effect relationship with the prescription of the amino-acid based formula), refusal to eat in 1 (11 month) child (implied reason – taste), black stool in 2 and bloating in 1 child. Treatment had to be discontinued in two cases: 1–due to the lack of effect and 2–due to financial constraints. 58 out of 60 children (97%) completed the diet course with a positive result and after 14–28 days were switched to the extensively hydrolyzed formula.

Conclusion: The use of the amino-acid based formula opened the prospect of fast, effective and safe treatment, as well as non-invasive and affordable diagnostics of cow's milk protein allergy in babies.

826

Effects of dietary counselling on protein intake and plasmatic protein profile in children with food allergy

D'Auria, E¹; Barberi, S¹; di Dio, F¹; Palazzo, S¹; Giovannini, M¹

¹Department of Pediatrics, University of Studies of Milan, San Paolo Hospital, Milan, Italy

Background: To investigate the protein intake and plasmatic protein profile in children with food allergy (FA) on elimination diet.

Method: The study included 40 children (19 boys, 3–36 months of age), all affected by FA on elimination diet for at least 30 days. Measurement of total protein, albumin, prealbumin and evaluation of plasma aminoacidogram (in order to calculate plasma nonessential to essential amino acid ratio) were carried out at enrollment (t_0) and after 6 months (t_1) of dietary counselling by registered dietician. 20 non allergic children (10 boys, 3–36 months of age) were enrolled at t_0 as control group to investigate protein intake in non allergic children. Dietary protein intake was assessed by a 3-day diary in all 60 children at t_0 and only in allergic children at t_1 .

Results: Protein intake in FA children exceeded the Italian recommended intake, according to national dietary recommenda-

tions, as in non allergic children. The ratio between the mean value of protein intake (g/kg) in allergic children and the recommended protein intake (g/kg) decreased significantly from t_0 to t_1 [$t_0 = 1.60$ (0.7–3), $t_1 = 1.31$ (0.9–2); $P = 0.05$] after nutritional counselling. There were no significant differences in protein intake between allergic and non allergic children at t_0 . The comparison of the measurements of plasmatic protein profile [mean (min-max)] observed in the allergic population during the two times of evaluation showed no significative difference between plasmatic total protein [$t_0 = 6.71$ g/dl (6.14–7.47), $t_1 = 6.82$ g/dl (5.88–7.52); $P = 0.076$], albumin [$t_0 = 4.56$ g/dl (4.14–5.14), $t_1 = 4.51$ g/dl (4.11–4.81); $P = 0.464$], prealbumin [$t_0 = 19.08$ mg/dl (13.40–24.40), $t_1 = 18.13$ mg/dl (13.70–24.50); $P = 0.241$]. However, it showed a significative difference between plasma nonessential to essential amino acid ratio [$t_0 = 2.35$ (1.20–4.33), $t_1 = 2.17$ (1.03–3.67); $P = 0.031$] with a significative increase of two essential aminoacids [Isoleucine: $t_0 = 52.99$ μ M (36.18–85.97), $t_1 = 56.41$ μ M (40.89–105.13); $P = 0.012$. Valine: $t_0 = 216.10$ μ M (129.98–385.42), $t_1 = 226.96$ μ M (161.02–427.17); $P = 0.018$].

Conclusion: An unbalanced diet is a frequent feature in allergic children, as well as in healthy children. This condition could be prevented and resolved by an adequate nutritional counselling. In our population the dietary intervention has been demonstrated to improve the quality of protein intake as shown by the decrease of the plasmatic non essential/essential aminoacids ratio.

827

Failure of peanut reintroduction in children

van Erp, FC¹; Boot, J¹; Knulst, AC²; Pasmans, SGM³; van der Ent, CK¹; Meijer, Y¹

¹Pediatric Pulmonology and Allergology, University Medical Center Utrecht, Utrecht, The Netherlands;

²Dermatology, University Medical Center Utrecht, Utrecht, The Netherlands;

³Pediatric Dermatology and Allergology, University Medical Center Utrecht, Utrecht, The Netherlands

Background: A negative food challenge (FC) should normally be followed by successful reintroduction of the challenged food. Several problems can occur; refusal of food that long has been avoided is a common problem in children. Failure of reintroduction could also indicate false negative FC outcome. This study describes reintroduction problems and risk factors for reintroduction failure in children.

Method: Parents of children with a negative peanut challenge in 2008–2010 were

invited for a telephonic interview up to three years after FC. The parents were questioned about the current diet, symptoms and problems during reintroduction and accidental reactions to peanut. Information about the history of peanut allergy and elimination diet and FC result was obtained from medical records. Successful reintroduction was defined as eating peanut or products containing peanut as ingredient on a regular basis.

Results: Follow-up data were obtained in 103 (94%) of children with a negative peanut challenge. In 33 (32%) of these children reintroduction failed. Refusal of products containing peanut was the reason of reintroduction failure in 15 (45%) children. In 11 (33%) children objective symptoms after the ingestion of peanut during reintroduction resulted in a prolonged elimination diet. A previous elimination diet for more than two years and a postponed reintroduction of more than 1 month after FC were significantly associated with reintroduction failure.

Conclusion: In children reintroduction failure after negative peanut challenge is common. Follow-up after FC should therefore be intensified to stimulate and monitor reintroduction and objectify accidental reactions. When symptoms after ingestion of peanut reoccur, revision of FC outcome should be considered.

828

Oral immunotherapy for treatment of egg allergy. Double blind placebo controlled study and post desensitisation follow-up

Caminiti, L¹; Porcaro, F¹; Chiera, F¹; Pajno, GB¹

¹Department of Pediatrics- Allergy Unit, University of Messina, Messina, Italy

Background: Hen's egg is common in infancy and currently avoidance of this food is the only approved treatment. We evaluated oral immunotherapy (OIT) using egg- white powder for the treatment of children with egg allergy. Once desensitisation was achieved a post- desensitisation follow-up was carried out.

Method: In this double blind, randomised placebo controlled study, 30 children 4–11 years of age with egg allergy received oral immunotherapy (17 children) or placebo (13 children). Initial dose escalation, build-up, and maintenance phases were followed by an oral food challenge with egg with powder and boiled egg. The children who achieved desensitisation (N. 16) did the withdrawal of egg for two months; afterwards these children underwent an oral food challenge with powder egg and boiled egg to test the tolerance.

Results: After about three months of OIT none of children who received placebo and 94% of the children who received powder egg, achieved desensitisation. Among 16 desensitised children, after two months of egg withdrawal: 65% achieved tolerance and 35% lost desensitisation and 'recalled' again food allergy. Of the immune markers measured small wheal diameters on skin- prick testing and increases in egg specific IgG4 antibodies levels were associated with both desensitisation and tolerance.

Conclusion: These results show that OIT can desensitise a very high proportion of children with egg allergy and induce in the majority of them (65%) a true tolerance.

829

The effectiveness and safety of specific oral immunotherapy in children with food allergy at stratification

Takahashi, M^{1,2}; Taniuchi, S¹; Soejima, K¹; Hatano, Y¹; Nakano, K¹; Kaneko, K¹

¹Pediatrics, Kansai Medical University, Osaka, Japan;

²Pediatrics, Izumiotsu Municipal Hospital, Osaka, Japan

Background: Although several clinical trials have been conducted on specific oral immunotherapy (SOIT), their effectiveness and safety has not been established thus far. We aimed to elucidate the effectiveness and safety of SOIT in children with food allergy who were stratified by age and the threshold dose in the food challenge test.

Method: In total, 149 children with food allergy (hen's egg, 67 children; wheat, 41 children, and cow's milk, 41 children), which were confirmed by the positive food challenge test, were enrolled in this study. The median ages of children with hen's egg, wheat, and cow's milk allergy were 6.2, 7.0, and 5.0 years at the start of the study (range 2.1–14.3 years, 1.3–12.3 years, and 2.7–14.3 years). The enrolled children were classified into two groups based on their age and threshold dose for food allergens at the food challenge test. Rush SOIT was performed in children aged more than 5 years using less than 1 g, 10 g, and 10 ml of the threshold dose of hen's egg, wheat, and cow's milk, respectively. Slow SOIT was performed in for the other children. The target dose for hen's egg, wheat, and cow's milk was set as 50 g, 200 g, and 200 ml, respectively.

Results: One year after SOIT, 79% of children with hen's egg allergy, 80% of those with wheat allergy and 93% of those with cow's milk allergy were able to consume the target dose of the respective foods. During SOIT, four patients required adrenaline treatment (total, 8 times) for serious reactions.

Conclusion: The study suggests that SOIT is effective and safe for children with food allergy at stratification.

830

Interest of a pocket scale used for oral immunotherapy at home

Asbati, Z¹; Dano, D¹

¹Department of Internal Medicine, Clinical Immunology and Allergology, University of Lorraine, Faculty of Medicine, Nancy, France

Background: Oral immunotherapy (OIT) is a major opportunity as a therapy of IgE-mediated food allergy. OIT protocols are generally established on several weeks or months using progressive doses starting with small doses below the threshold dose determined by a Double Blind Placebo Controlled Food Challenge (DBPCFC). In food laboratory, the preparation of OIT protocols especially dose weighing is sometimes time-consuming. On the other hand home weighing can be difficult and inaccurate particularly for small doses (less than 100 milligram).

Method: In order to facilitate the establishment of OIT at home, we set up an individual pocket scale from *OHAUS YA Series* (model: *YA102*) with a precision ranging from 0.01 to 100 g. The efficiency of the scale was checked by a metrology professional. OIT using the scale was proposed to a 13 years old patient with a persistent food allergy to hazelnut. According to patient's clinical data, the protocol was established on 24 weeks period of time; with increasing doses from 50 mg to 10 g of raw hazelnut flour in the end of the protocol.

An emergency kit was given and instructions for use explained to the patient. A satisfaction questionnaire was filled by the patient allowing the evaluation of this procedure for immunotherapy application, as well as to evaluate the convenience for patients self-use at home.

Results: The patient completed with success the protocol and no severe reaction has occurred during the desensitisation period. Practically the protocol was clear and the daily weighing was easy, the patient confirmed the facility of use and transportation of the scale.

The results of the questioner support the idea of the scale's utility for OIT daily employ at home.

Conclusion: Numerous studies are supporting the interest of immunotherapy to improve the security and quality of life of the allergic patient and his family. The therapeutic education of the patient based on home weighing using an individual pocket scale can be a solution in order to reduce difficulties related to OIT protocol's management.

831

The influence of IgE-mediated cow's milk allergy to cow's milk consumption in childhood

Mitropoulou, K¹; Politis, P²; Trigka, M¹

¹Paediatric Allergy Unit, Dept of Paediatrics, General University Hospital of Patras, Patras, Greece; ²General Hospital of Aegion, Aegion, Greece

Background: Cow's milk (CM) represents a major source of high nutritional quality protein as well as of calcium and is of profound significance for bone metabolism. In developed countries CM optimal daily intake should be around 500 ml, adequately complemented with other nutrients.

Aim: To evaluate whether children with a history of IgE-mediated cow's milk allergy (CMA), who have outgrown it, consider CM acceptable and consume similar daily quantities to non-CMA controls.

Patients and methods: Subjects were children between 3.8 and 13.5 years diagnosed with CMA in the first year of life. Diagnosis of CMA was based on history, clinical findings and CM-specific IgE status, detected via skin prick test and/or CAP system technology. Healthy siblings 4–17 years, without any food allergy, were selected as controls. Mothers were contacted via telephone and asked to indicate their children's preference for CM and if they consume <400 ml or ≥400 ml/day.

Results: Forty-three subjects (mean age 7.24 ± 2.55 years, 39.5% boys) and 48 controls (mean age 8.15 ± 3.15 years, 50% boys) participated in the study. Twenty-six of 43 subjects (60.6%) reported dislike for milk and drinking <400 ml/day, compared with 7 of 48 controls (14.6%). Chi-square test = 20.6 $P < 0.001$ [odds ratio 8.96; 95% confidence intervals 3.27–24.55].

Conclusion: A significant proportion of subjects with CMA in infancy dislike CM and consume sub-optimal amounts in childhood. We propose that early taste

experiences through CMA *per se* or the milk hydrolysate formulas influence the children's subsequent preferences.

832

Comparison of clinical characteristics of oral allergy syndrome between children grouped according to age

Matsuura, M¹; Inomata, N¹; Nomura, Y¹; Aihara, M¹

¹Department of Environmental Immuno-Dermatology, Yokohama City University School of Medicine, Yokohama, Japan

Objective: To evaluate the clinical characteristics of oral allergy syndrome (OAS) according to age in children.

Method: Children aged 10 years or under with a diagnosis of OAS were divided into two groups according to age and analyzed with regard to causative foods and IgE levels for pollen allergens, including allergic components, such as rBet v 1 and rBet v 2, which have been known to be cross-reactive to plant-derived foods, measured by ImmunoCAP.

Results: Group A (under 6 years of age) included four cases (mean age 3.3 years, M:F = 1:3) and Group B (age 6 years or over) included seven cases (mean age 7.3 years, M:F = 5:2). The mean level of total IgE was 1264 IU/ml and 834 IU/ml in Groups A and B, respectively. The frequent causative foods were kiwi ($n = 2$) and cashew nuts ($n = 2$) in Group A whereas apples ($n = 4$), strawberries ($n = 3$), peaches ($n = 3$), and melons ($n = 3$) were causative foods in Group B. The prevalence of atopic dermatitis and pollinosis was 75% and 0% in Group A and 57.1% and 85.7% in Group B, respectively. Positivity for specific IgE for pollen allergens was 25% and 100% in Groups A and B, respectively. The pollen allergens sensitised were orchard grass ($n = 1$) in Group A and alder ($n = 7$), cypress ($n = 7$), and orchard grass ($n = 5$) in Group B. Furthermore, in three cases in Group B, positivity for specific IgE for rBet v 1 and rBet v 2 was 100% and 33.3%, respectively.

Conclusion: Although there is the limitation of the small number of subjects in the current study, the results indicated that OAS in most Group B cases, that is, the older age group, could be due to cross-reactivity to pollens, a finding not observed in Group A.

Poster Session 26

Diagnosis of food allergy I

833

Component resolved analysis of sera from peanut sensitised patients reported to the Norwegian Food Allergy Register show age related sensitisation profiles

Namork, E¹; Stensby, BA¹; Løvik, M^{1,2}

¹Dept. Environmental Immunology, Norwegian Institute of Public Health, Oslo, Norway; ²Norwegian University of Science and Technology, Trondheim, Norway

Background: Peanut is a common and potent allergen and is the leading cause of fatal allergic reactions to food worldwide. The prevalence of peanut allergy appears to be increasing, especially in children. The National Register of Severe Allergic Reactions to Food was established in 2000 with the purpose of gaining information about food allergy in Norway. Cases of patients allergic to peanut reported to the Food Allergy Register amount to about 20%, which is the highest cause of severe allergic reactions reported.

Method: Analysis using recombinant peanut allergens allows differentiation between severe, persistent primary peanut allergy and cross-reactivity due to primary allergy to pollen that may cause oral allergy syndrome (OAS). Specific IgE reactivity to the stable storage proteins Ara h1, 2/6 and 3, lipid transfer protein (LTP) Ara h9 and the labile birch pollen homologue Arah8 were tested in 95 sera positive to whole peanut extract.

Results: The majority of the 95 sera contained IgE to Ara h8 (55%). Sensitisation to the storage proteins Ara h1, 2/6 and 3 were found in 22%, 28% and 22%, respectively, while 11% were sensitised to LTP Ara h9. The age distribution showed two peaks; one group of children aged 2–15 years and one adult group 30–40 years old. Examination of the two age groups separately, showed that 70% of the children were sensitised to the storage proteins Ara h1, 2/6, and 3 or to Ara h9 in different combinations and 21% were positive to both Ara h8 and birch. A few sera were negative to all the peanut components and birch, but positive to timothy. In the adult group, only 13% were sensitised to the storage proteins or to LTP. A majority of 64% was sensitised to both Ara h8 and birch, while 23% were negative to all the components, but positive to timothy pollen and/or nuts like cashew and walnut.

Conclusion: Component analyses of sera from peanut allergic patients show two age groups with different sensitisation profiles. Specific IgE mainly to the stable peanut components indicates increased risk of severe peanut allergy for children up to 15 years of age. Oppositely, the result for the adult group indicates primary pollen allergy to mainly birch but also some to timothy or nuts causing cross-reactivity to peanut. Patients suffering from peanut allergy will benefit from this information since cross-reactions are less often associated with systemic reactions but more often with milder and local symptoms like OAS.

834

Clinical relevance of sensitisation to profilin in Japanese patients with plant food allergy

Minami, T¹; Fukutomi, Y¹; Taniguchi, M¹; Saito, A¹; Yasueda, H¹; Nakayama, S²; Tanaka, A²; Mitsui, C¹; Hayashi, H¹; Tsuburai, T¹; Mori, A¹; Hasegawa, M¹; Akiyama, K¹

¹Clinical Research Center for Allergy and Rheumatology, Sagami National Hospital, Sagami, Kanagawa, Japan; ²Phadia AB (Thermo Fisher Scientific), Uppsala, Sweden

Background: Profilin is a cross reactive pan allergen that induces pollen-food allergy syndrome. However, the clinical relevance of profilin in IgE cross-reactivity is still controversial, and pollen as an allergen source contributing to profilin sensitisation has not been established.

Objective: To identify the source pollen causing profilin sensitisation and elucidate the clinical relevance of profilin as a food allergen in the Japanese population.

Method: Sixty-nine Japanese patients with plant food allergy were studied in terms of their sensitisation to various allergen components using ImmunoCAP ISAC (Phadia). Patients sensitised to Phl p 12 (timothy profilin, P+ patients) were compared with those not sensitised to Phl p 12 (P- patients), in term of their cosensitisation to other allergen components, types of offending food, number of offending foods, and symptoms provoked after eating such offending foods.

Results: Among 69 patients, 19 patients (28%) were sensitised to Phl p 12. P+

patients were more likely than P- patients to be sensitised to Cyn d 1 (89% vs 46%, $P = 0.01$), Phl p 1 (79% vs 44%, $P = 0.009$), and Phl p 5 (42% vs 18%, $P = 0.042$), which are diagnostic marker allergens for identifying genuine grass-pollen-sensitised patients. On the other hand the prevalences of sensitisation to Bet v 1, Amb a 1, and Art v 1 were not different between the two patient groups. Allergic symptoms were more frequently triggered by melon, tomato, eggplant and banana in P+ patients than P-patients. The number of causal foods was larger in P+ patients than in P- patients, indicating that profilin sensitisation is related to a broader clinically relevant cross-sensitisation to plant foods. P+ patients were more likely to experience diarrhea, lower abdominal pain, and unexpected food-related allergic symptoms.

Conclusion: These findings suggest that, in this study population, the main source of profilin sensitisation was grass pollen, and profilin sensitisation was related to clinically relevant cross-sensitisation to plant-derived foods.

835

Accuracy of alcohol-dissolved wheat extract for the diagnosis of IgE-mediated wheat allergy

Pacharn, P¹; Siripattanamongkol, N¹; Pannakapitak, N¹; Visitsunthorn, N¹; Jirapongsananuruk, O¹; Vichyanond, P¹

¹Pediatrics, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background: Wheat allergy is a common allergic condition in Asia, including Thailand. Since wheat allergens are alcohol-soluble, commercially water-soluble may not be sufficient for the diagnosis of wheat allergy. We sought to determine the accuracy of in-house, alcohol-dissolved wheat extract in Thai children with immediate type wheat allergy.

Method: Thirty children (aged from 15 months to 13 years) with history of immediate reaction to wheat underwent skin tests with commercial and in-house wheat extract (dissolved wheat with alcohol 70%) along with determination of specific IgE for wheat and omega-5 gliadin. Eighteen patients underwent open

challenge with 25 gm of wheat. Twelve patients who had history of wheat anaphylaxis were considered wheat allergic and did not undergo challenging. The receiver-operator characteristic (ROC) curves and predictive decision points of skin prick tests and specific IgE were determined.

Results: Eleven out of 18 children (61%) had positive result for wheat challenge test. The in-house wheat extract has a good correlation with commercial wheat extract ($r = 0.89$). The wheal size of 3 mm for both in-house wheat extract and commercial wheat extract gave the best diagnostic value (AUC 0.92, and 0.88, respectively). However, at these criteria, the in-house wheat extract gave sensitivity 91.3%, specificity 71.4%, PPV 91.3%, and NPV 71.4%, whereas commercial extract gave sensitivity 73.9%, specificity 100%, PPV 100%, and NPV 53.8%. The accuracy of commercial wheat extract, in-house wheat extract, specific IgE for wheat, and omega-5 gliadin were 80%, 86.7%, 86.7%, and 80%, respectively. ($P = 0.03$).

Conclusion: Using in-house, alcohol-dissolved wheat extract for skin prick test appeared to produce improved accuracy for diagnosing immediate type wheat allergy, compared to commercial extract.

837

Monitoring of heart rate variability during oral food challenge could improve patient safety and diagnostic yield

Twomey, N¹; Temko, A¹; Cullinane, C²; Daly, D²;

Marnane, WP¹; Hourihane, JO²

¹University College Cork, Electronic and Electrical Engineering, Cork, Ireland; ²University College Cork, Paediatrics and Child Health, Cork, Ireland

Background: International consensus is that an Oral Food Challenge (OFC) should continue until objective, predetermined clinical stop criteria are met. OFC can cause anaphylaxis. Anaphylaxis appears more common late in OFC, after a large cumulative dose of test allergen, rather than early in OFC after very small doses. Heart rate variability (HRV) is noted before the clinically observable onset of sepsis and seizures in hypoxic ischaemic encephalopathy and other immune mediated disorders.

Method: We retrospectively examined HRV, using fully automated computer-based detection of ECG signals, during 24 clinically-indicated open OFC. Baseline HRV was examined before the 1st dose of OFC was given and epochs of HRV during OFC were compared to this baseline, using 18 known HRV factors. OFC was stopped or continued to top dose, according to clinical practice, blind to the HRV data.

Results: Fifteen OFC were positive, 9 were negative. HRV was stable in all negative OFC (100% specificity) and HRV changes were noted in 12/15 positive OFC (80% sensitivity). Use of HRV detection during OFC could have led to 70% reduction in cumulative doses administered during positive OFC.

Conclusion: Detection of complex features of Heart Rate Variability during oral food challenge can be automated at the bedside. This finding has potential to widen the adoption of diagnostic OFC, by reducing premature termination of OFC due to subjective symptoms and by improving safety in positive OFC by prompting termination of positive OFC at lower doses of test allergen.

838

Specific IgE does not predict allergy to specific species within an adult fish allergic population

Schulkes, K¹; Klemans, R¹; Knigge, L¹; de Bruin-Weller, M¹; Bruijnzeel-Koomen, C¹; Marknell DeWitt, A²;

Lidholm, J²; Knulst, A¹

¹Dermatology/Allergology, University Medical Center Utrecht, Utrecht, The Netherlands; ²Thermo Fisher Scientific, Uppsala, Sweden

Background: Fish is an important cause of food allergy. Studies on fish allergy are scarce and in most cases limited to serological evaluation. Our objective was to study patterns of both clinical allergy and sensitisation to 13 commonly consumed fish species.

Method: Thirty-eight adult patients with allergy to different fish species completed a questionnaire regarding general allergy characteristics, age of onset of fish allergy and symptoms to 13 fish species. Patients were included if they reported symptoms after ingestion of fish in combination with a positive challenge or sensitisation; a sIgE level to cod ≥ 0.35 kU_A/l and/or a positive skin prick test (SPT) to at least one of the tested fish species. Specific IgE to all 13 fish species were analyzed by ImmunoCAP.

Results: Median age of onset of fish allergy was 8.5 years. Severe reactions were reported by the majority of patients (42% respiratory, 16% cardiovascular symptoms). After diagnosis, 66% of patients eliminated all fish. Allergy to all 13 species was reported by 59%. Cod (84%) and herring (79%) were the most frequently reported culprit species while tuna (58%), hake (57%) and swordfish (55%) were the least frequent. Among those who reported symptoms to a particular species, the frequency of detectable sIgE to the same species ranged between 50% (swordfish) and 100% (hake). Nega-

tive sIgE results among those who reported tolerance ranged from 0% (hake, pollock and swordfish) to 75% (sardine). For cod, the agreement between sIgE test results and reported symptoms or tolerance was 82% and 25%, respectively. Sensitisation to cod parvalbumin (Gad c 1) was present in 77% of all patients.

Conclusion: Serological cross-reactivity between fish species is frequent, but clinical relevance appears limited to some species. Cod and herring are the most frequent causes of fish allergy. A well-taken history or food challenge is required for discrimination between allergy to different fish species.

839

Clinical evaluation of component sensitisation in children and young adult with peanut allergy

Kitabayashi, T¹; Adachi, M²

¹Pediatrics, International University of Health and Welfare Mita Hospital, Tokyo, Japan; ²Sanno Hospital, Tokyo, Japan

Background: We confirmed there are two patterns of peanut allergy in our previous study. The purpose of the study is to investigate sensitisation pattern to peanut components between peanut allergy and peanut sensitised patients.

Methods: Specific IgE antibodies to five peanut components in sera from patients referred to our clinic between 2011 and 2012 were measured by ImmunoCAP. Forty-five children and young adults with suspected peanut allergy were enrolled in the study. Fifteen of those (1.1–20.5 years) were Oral Allergy Syndrome (OAS) after ingestion of peanut. Twenty-two (1.5–15.4 years) were immediate systemic (IM) type of peanut allergy. Eight were peanut sensitised children without allergic symptom to peanut (NoPA).

Results: IM type had significantly higher levels of specific IgE to Ara h 2 comparing to OAS type and NoPA. Patients of OAS type were frequently sensitised to PR-10 and LTP (Ara h 8: 47%, Ara h 9: 40%) comparing to IM type. NoPA were sensitised high positive rate to Ara h 3 compared with IM patients and OAS type (IM:55%, OAS: 20%, NoPA: 75%). OAS type were frequently sensitised to other food and pollen components (Cor a 1, Cor a 8, Ber e 1, Gly m 4, Bet v 1) comparing to IM type. There is a high correlation among Bet v 1 and Ara h 8, Cor a 1, Gly m 4. But there is no correlation between Ara h 9 and Cor a 8.

Conclusion: The results indicated that there are characteristic pattern of sensi-

sation and concentration by clinical entity. Allergen component testing is a useful in identifying peanut allergy.

840

Serological characterisation of milk allergic patients – can severity be predicted by components?

Petersen, TH¹; Eller, E¹; Mortz, CG¹; Bindslev-Jensen, C¹
¹Department of Dermatology and Allergy Center, Odense Research Center for anaphylaxis, Odense University Hospital, Odense, Denmark

Background: Cow's Milk Allergy (CMA) is one of the most frequent allergies in infancy affecting approximately 2% of children. The majority will outgrow it before the age of 4. The oral food challenge is the gold standard for diagnosing CMA but is time consuming and costly.

Component resolved diagnostics by measuring IgE to specific milk proteins (components) are reported to be promising as replacement for the oral food challenge in other types of food allergies (e.g. peanuts).

The aim of this study is to evaluate components and their ability to: 1) Predict the outcome of oral challenge in CMA. 2) Distinguish patients outgrowing their CMA from patients with persistent CMA. 3) Predict the severity of the allergic reaction.

Method: Data from 85 patients followed at the Allergy Center, Odense University Hospital, Denmark, were retrospectively evaluated. Forty-six patients had a negative first challenge and 39 one or more positive challenges. Of these 26 patients developed tolerance to milk (negative challenge). Challenges were performed according to EAACI guidelines. s-IgE to cow's milk and cow's milk components (Lactoferrin, α -lactalbumin, β -lactoglobulin and Casein) were measured with ImmunoCap (Thermo-Fisher Diagnostic).

Results: We found a strong correlation ($r = 0.98$, $P < 0.01$) between s-IgE and the sum of the four measured components. We were not able to differentiate between positive and negative challenges neither by s-IgE nor by the four measured components. The subgroup of children with persistent CMA after 8 years of age had significantly higher levels of s-IgE, α -lactalbumin, β -lactoglobulin and casein ($P < 0.01$) than the ones with a negative challenge.

We found for all positive challenges a correlation between the levels of s-IgE to cow's milk, the components and the severity of the allergic reaction during oral food challenge, although with large individual variations.

Conclusion: Our results indicate that IgE antibodies to milk protein components are not able to replace oral challenges in the

diagnosis of cow's milk allergy or to distinguish patients outgrowing their CMA from patients with persistent CMA. The levels of s-IgE to cow's milk correlate to the severity of the allergic reaction when performing food challenges.

842

IgE and IgG₄ antibody levels towards milk components are associated with outcome of oral immunotherapy in Finnish milk allergic children

Englund, H¹; Kuitunen, M²; Movérare, R^{1,3}; Borres, MP^{1,4}; Mäkelä, M²
¹Thermo Fisher Scientific, Uppsala, Sweden;
²University of Helsinki, Skin and Allergy Hospital, Helsinki, Finland; ³Department of Medical Sciences, Respiratory Medicine and Allergology, Uppsala University, Uppsala, Sweden; ⁴Department of Pediatrics, Institute for Clinical Sciences, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

Background: IgE-mediated cow's milk allergy (CMA) is a common food allergy in young children. The majority of children outgrow CMA, but for approximately 20 percent the symptoms persist beyond the age of five. For these children, the use of oral immunotherapy (OIT) to induce tolerance is an option.

Objective: To investigate IgE and IgG₄ antibody levels to milk and milk allergen components before and after OIT, and relate it to clinical outcome of OIT.

Methods: Finnish children ($n = 81$) above six years of age and with persistent CMA were subjected to a six months OIT protocol (according to Meglio et al, Allergy, 2004). The target dose was tolerance of 200 ml milk. Patients were food challenged, carefully examined and blood samples were collected before and after OIT. IgE and IgG₄ antibody levels towards milk, α -lactalbumin, β -lactoglobulin, casein, lactoferrin and bovine serum albumin were analyzed with ImmunoCAP. The sample material was divided into two groups, those who reached the 200 ml target dose ($n = 66$) and those who did not ($n = 15$).

Results: OIT was successful in 81% of the patients (66/81). Patients who did not reach the 200 ml target dose (15/81) showed, as a tendency, higher concentrations of IgE towards β -lactoglobulin and casein before start of treatment. The 81% who reached the target dose during OIT had a larger increase in IgG₄-levels towards α -lactalbumin, β -lactoglobulin, casein and lactoferrin during treatment, than those with only partial or no tolerance.

Conclusion: Measurement of IgE and IgG₄ antibody levels to milk components may be useful for predicting outcome and moni-

toring therapeutic efficacy when treating milk allergic children with oral immunotherapy.

843

Cross-reactivity between peach (*Prunus persica*) and *Phleum pratense*, *Olea europaea* and *Parietaria judaica* pollen

Abel-Fernández, E¹; Fernández-Caldas, E¹; Cases, B¹; Tudela, JI¹; Esteban López, MI²; Casanovas, M¹; Subiza, JL¹
¹Research and Development, Immunotek S. L., Alcalá de Henares, Spain; ²Hospital General de Segovia, Segovia, Spain

Background: Cross-reactivity frequently occurs among pollens and between vegetables and pollens. In Spain, Rosaceae fruits induce 70% of food plant allergies, being peach (*Prunus persica*) the most important allergenic fruit. Grass (*Phleum pratense*), olive (*Olea europaea*) and wall pellitory (*Parietaria judaica*) also present high rates of sensitisation. Cross-reactivity between pollen and food plants has important clinical implications. The aim of the present study was to analyze *in vitro* cross-reactivity between peach and *Phleum*, *Olea* and *Parietaria* pollen extracts.

Method: Nineteen patients (mean age 25.89 ± 11.18) showing clinical symptoms of respiratory and food allergy and positive cutaneous and immuno-CAP tests to peach or related fruits, grass, olive and/or wall pellitory were evaluated; six sera were selected based on high specific IgE levels to these allergens. Specific IgE to the extracts and to individual allergens was determined by ELISA, immunoblots and ISAC. Allergenic extracts of peach peels, grass, olive and wall pellitory pollens were also analyzed by electrophoretic profile determination and protein identification (LC-MALDI MS/MS). Cross-reactivity was evaluated by ELISA inhibition assays.

Results: Inhibition assays showed cross-reactivity between peach and the three pollen species analyzed. The analysis of the allergen extracts revealed the presence of the major allergens, as well as other allergenic and non-allergenic proteins and pan-allergens.

Conclusion: Significant cross-reactivity between peach and *Phleum*, *Olea* and *Parietaria* was detected using the serum of peach and pollen allergic patients. The role of major and minor allergens and of allergens with a high molecular weight should also be considered.

844

Allergy to fish and cross-sensitivity between fish species

Witten, M¹; Malling, H-J¹; Bindeslev-Jensen, C²; Schnoor, HJ¹; Poulsen, LK¹

¹Dermato-allergological Department K, Allergy Clinic, Gentofte University Hospital, Hellerup, Denmark;

²Dermato-allergological Department, Allergy Center, OUH, Odense, Denmark

Background: Fish allergy is believed to be present in approximately 0.4 percent of the adult population in Denmark and is suspected to be a lifelong condition. It is common practice that patients are tested for codfish and if positive the patients are advised to avoid all fish types according to the assumption that there is a considerable amount of cross-sensitivity between the different fish species. There is, however an increasing number of patients, who report being allergic to a different fish species than codfish, or are allergic to codfish, but can tolerate other species. The aim was to examine fish allergic patients as to which species they react towards and to examine whether Standard Prick Test (SPT), specific IgE or Histamine Release (HR) are sufficient to diagnose allergy to both codfish and other fish species, defined by a positive food challenge (DBPCFC).

Method: Up to 40 patients age 18–65, suspected of fish allergy were tested with SPT and IgE for 19 different fish species and Anisakis simplex. Eight patient suspected of fish-allergy were challenged with double-blinded, placebo-controlled food challenges (DBPCFC) with at least three different fish-types: codfish, tuna and salmon. Exclusion criteria were pregnancy, significant other disease or betablockers.

Results: DBPCFC's in eight patients showed that for two patients no culprit fish could be identified. For the six patients with a reaction in the DBPCFC only three reacted classically towards codfish. The other three patients were species-specific and did not react to codfish however they reacted to tuna, salmon or herring respectively. For the SPT the sensitivity was 100 percent however five patients had a positive test without a matching positive challenge. For the specific IgE two patients had a positive challenge towards a fish-type without increased IgE.

Conclusion: There is a need for food challenges of fish allergic patients with different fish-types according to the medical history, since many patients are only being tested with SPT or IgE for one or two fish types, and there is increasing evidence, that not all species cross-react. Particularly patients who have never been challenged or challenged several years ago can have been misdiagnosed or have outgrown their allergy. SPT showed high sensitivity and

low specificity which was expected, however both specific IgE and HR showed both low sensitivity and specificity indicating that the food challenge is of great importance.

845

Unespected isolated IgE reactivity to native walnut vicilin-like protein (nJug r2) on ISAC microarray: is it clinically relevant or due to cross-reactive carbohydrate epitopes?

Conte, M¹; Asero, R²; Martelli, P¹; Da Re, M¹; Stella, S¹; Villalta, D¹

¹Allergy and Clinical Immunology, A.O. S. Maria degli Angeli, Pordenone, Italy; ²Allergy Unit, Clinica San Carlo, Paderno Dugnano, Italy

Background: Since the introduction of the last version of the microarray-based ImmunoCAP ISAC 112™, three native walnut (*Juglas regia*) molecules are tested: 2S albumin (nJug r1), vicilin (nJug r2) and lipid transfer protein (nJug r3). In the daily practice we found many unespected cases of isolated sIgE reactivity to nJug r2: the aim of this study is to evaluate if this reactivity is clinically relevant or due to CCDs (cross-reactive carbohydrate determinants) positivity.

Method: Sera from 320 consecutive outpatients afferring to our Allergy Unit tested by ImmunoCAP ISAC 112™ from december 2011 to december 2012 were considered for the study. All nJug r2 positive patients were reviewed to assess allergy or tolerance to walnut. A linear regression analysis was performed to evaluate the correlation between nJug r2 and CCDs (nMUXF3) sIgE values and a CAP inhibition assay was used to confirm the possible cross-reactivity between the two molecules.

Results: 11.6% of the 320 collected sera (37) were positive to nJug r2. 8.1% (3) and 21.6% (8) were positive to nJug r1 and nJug r3 respectively. 73% (27) sera showed an isolated nJug r2 reactivity. Walnut allergy was clinically relevant only in nJug r1 positive patients. 67% (25/37) nJug r2 positive sera were simultaneously positive to nMUXF3 and showed a significant correlation ($P < 0.001$) in IgE levels between nJug r2 and nMUXF3 ($r^2 = 0.787$). A complete IgE reactivity inhibition to both nJug r2 and nMUXF3 was showed after incubating a nJug r2 positive serum with nMUXF3.

Conclusion: The unespected isolated sIgE reactivity to nJug r2 revealed by ImmunoCAP ISAC 112™ is clinically irrelevant and frequently due to reactivity to cross-reactive carbohydrate epitopes.

846

Comparison of skin prick test and specific IgE to hazelnut extract, Cor a 1 and Cor a 8 in predicting clinical reactivity in children with hazelnut allergy

Buyuktiryaki, B¹; Cavkaytar, O¹; Sahiner, UM¹; Arik Yilmaz, E¹; Yavuz, ST¹; Soyer, OU¹; Tuncer, A¹; Sackesen, C¹

¹Department of Pediatric Allergy, Hacettepe University Faculty of Medicine, Ankara, Turkey

Background: Hazelnut allergy is the most common nut allergy in Turkish children. The cross-reactivity between hazelnut Cor a 1 and Betula Bet v 1 is well-known in Europe and USA and this cross-reactivity was shown to increase the level of IgE to Cor a 1. Recently, a lipid transfer protein, Cor a 8, was shown to be responsible for the severe reactions. Our aim was to determine the cut-off levels of diagnostic tests to predict clinical reactivity and to investigate the cross-reactivity of hazelnut allergens with pollens.

Method: Based on the presence of hazelnut SPT ≥ 3 mm and sIgE > 0.35 kU/l, 56 children who underwent DBPCFC and 8 children who experienced anaphylaxis in the last 6–12 months were included. Serum IgE levels to hazelnut extract, Cor a 1, Cor a 8, and Bet v 1 were measured. Wheal sizes and sIgE values were analyzed by ROC curves.

Results: The median [interquartile range (IQR)] age of 64 children was 3.4 (2.1–7.2) years. Twenty-four of 56 DBPCFC tests were positive, 10 children had anaphylaxis at DBPCFC test and 8 children had anaphylaxis after accidentally hazelnut intake. The cut-off hazelnut sIgE level that predicts clinical reactivity with 75% sensitivity and 90.6% specificity was found 3.15 kU/l. The cut-off hazelnut skin prick test wheal size that predicts clinical reactivity at the highest sensitivity (59%)+ specificity (90%) was 7.5 mm. Multivariate logistic regression analysis revealed hazelnut prick test wheal size as a significant risk factor for anaphylaxis (OR:1.4, 95%CI:1.0–2.1, $P = 0.028$). Thirty children (55.6%) had concomitant aeroallergen sensitisation. The median of IgE to Cor a 1 was 0.01 (0–0.03) (max = 0.15). The median of IgE to Bet v 1 was 0.03 (0.01–0.05), all results were under 0.39 kU/l. In children older than 2 years of age, IgE to Cor a 8 was significantly higher in children with grass pollen sensitisation [0.21 (0.05–2.47)] compared to children without sensitisation [0.02 (0–0.08)] ($P = 0.001$).

Conclusion: This is the first study denoting the cut-off value of hazelnut sIgE to predict clinical reactivity with a history suggestive for hazelnut allergy in children. Skin prick test reactivity revealed more

reliable marker than hazelnut sIgE in the case of predicting anaphylaxis. Our results show that *Betula* sensitivity is not seen in Turkish children with hazelnut allergy but grass pollen sensitisation appears to influence the level of IgE to Cor a 8.

847

Diagnostic utility of IgE antibodies to omega-5 gliadin and three other gluten derived wheat components in wheat allergic children

Nilsson, N¹; Sjölander, S²; Baar, A³; Pahr, S³; Valenta, R⁴; Vrtala, S⁵; Morita, E⁶; Berthold, M²; Hedlin, G¹; Borres, M⁶; Nilsson, C⁷

¹Q204 Lung/Allergy Department, Karolinska University Hospital, Stockholm, Sweden; ²ImmunoDiagnostics, Thermo Fisher Scientific, Uppsala, Sweden; ³The Christian Doppler Laboratory for the Development of Allergen Chips, Medical University of Vienna, Vienna, Austria; ⁴Division of Immunopathology, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria; ⁵Department of Dermatology, Shimane University Faculty of Medicine, Shimane, Japan; ⁶Department of Pediatrics, Institute for Clinical Sciences, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden; ⁷Department of Clinical Science and Education, Karolinska Institutet and Sachs Children's Hospital, Stockholm, Sweden

Background: One common food causing allergy in children is wheat. IgE-antibodies (IgE-ab) to wheat have been reported to correlate poorly to the outcome of oral wheat challenge tests. Wheat contains many allergens and one of them is ω -5 gliadin. IgE-ab to ω -5 gliadin is associated with WDEIA in adults and immediate wheat allergy in children. IgE-ab to other wheat components in relation to wheat allergy has not been studied in detail.

Aim: To correlate IgE-ab to ω -5 gliadin and three other gluten derived allergen components with the outcome of oral wheat challenge in children with a diagnosis of wheat allergy.

Method: Sixty-three children (0–18 year) with a doctor's diagnosis of wheat allergy, IgE-ab to wheat and on a wheat elimination diet, were subjected to an open oral wheat challenge with doses from 0.05 g to 17 g of white bread. The children were observed during, and up to two hours after the provocation and the severity of allergic symptoms were scored according to Astier from 0 to 5 while a negative test was defined as no objective allergic symptoms. Blood samples were taken before the challenge and were analysed for IgE-ab using ImmunoCAP to recombinantly produced ω -5 gliadin, low molecular weight glutenin (LMW-glutenin), high molecular weight glutenin (HMW-glutenin) and a native gliadin preparation containing α , β , γ , and ω -5 gliadin.

Results: Thirty-one children had a negative challenge and 32 had a positive challenge.

Thirteen had score 1, four score 2 and fifteen score 4. The IgE-ab levels to all four allergen components were significantly higher in the challenge positive compared to the challenge negative children ($P < 0.0001$). In the challenge positive group 21/32 had IgE-ab to ω -5 gliadin, 25/32 to LMW-glutenin, 30/32 to gliadin and 31/32 to HMW-glutenin. The 11 children that were negative to omega-5 gliadin all had IgE-ab to one or more of the three other components. Children with symptom score 4 had significantly higher IgE levels specific for all four components compared to children with less severe symptoms ($P < 0.05$).

Conclusion: Analysis of IgE-ab to wheat components derived from the gluten fraction may increase the ability to identify children suffering from wheat allergy. In this study analysis of IgE-ab to three gluten derived wheat allergens supplemented the analysis of IgE-ab to omega-5 gliadin as all wheat allergic children had IgE-ab to one or more of the four components.

848

Examination of 47 cases' provocation tests with food-dependent exercise-induced anaphylaxis

Asaumi, T¹; Yanagida, N¹; Iikura, K¹; Koike, Y¹; Okada, Y¹; Ogura, K¹; Shukuya, A¹; Ebisawa, M¹
¹Department of Allergy, Sagami National Hospital, Kanagawa, Japan

Background: Little has been reported regarding standardised methodology of provocation tests with food-dependent exercise-induced anaphylaxis (FEIA). In the Japanese Pediatric Guideline for Oral Food Challenge in Food Allergy 2009 (the Japanese OFC guideline), premedication of aspirin prior to challenge test is recommended. We examined the usefulness, the positive rate, and the safety of them.

Methods: A retrospective chart view was performed on children who underwent provocation tests with FEIA in our hospital from 2006 to 2012. Ergometer stress tests were administered after taking suspected foods. Aspirin was used based on the Japanese OFC guideline.

Results: A total of 210 tests were performed for 47 cases. We diagnosed 23 cases as definite FEIA out of 47 cases. Of the 210 tests, 37 tests (18%) evoked objective symptoms. It occupied 59% in the positive tests that symptoms were induced by more than 1 food. Causative foods were as follows; wheat four cases, combination of wheat and shrimp four cases, peach case cases, tangerine two cases, combination of wheat and apple two cases. Within 60 min from the beginning of exercise, 97%

evoked symptoms. Of patients who failed tests, there were 78% cutaneous, 27% lower respiratory, 8% gastrointestinal, 14% pharyngolaryngeal, 19% eye, 11% upper respiratory, 8% cardiovascular, 3% neurological reactions, and then 7% involved more than 1 organ system. Of 37 positive tests, we treated 32% of them, and administered adrenaline in 19% of cases. After discharge from hospital, negative cases had no symptoms of FEIA by foods which were provoked, but some were differently diagnosed as chronic urticaria or bronchial asthma.

Conclusions: Though provocation tests with FEIA sometimes induced severe symptoms, they were performed almost safely in admission. Half cases could lead to definite diagnosis, and the other half cases to exclusive diagnosis, thus FEIA provocation tests with aspirin were very useful for diagnosis and daily life guidance of patients.

849

Ara h 2 is the best predictor for peanut allergy in both children and adults but cannot be used to accurately exclude peanut allergy in an adult population

Klemans, R¹; Broekman, H¹; Knol, E^{1,2}; Bruijnzeel-Koomen, C¹; Otten, H²; Pasmans, S^{1,3}; Knulst, A¹
¹Dermatology/Allergology, University Medical Center Utrecht, Utrecht, The Netherlands; ²Immunology, University Medical Center Utrecht, Utrecht, The Netherlands; ³Center for Paediatric Allergology, Wilhelmina, Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands

Background: Specific IgE (sIgE) to Ara h 2 as clinical predictor for peanut allergy in children has a diagnostic value comparable to a prediction model containing gender, skin prick test (SPT), sIgE to peanut extract and total IgE minus sIgE. In adults, the diagnostic value of peanut components has not been studied yet. The aim of our study was to validate a pediatric prediction model in an adult population. In addition, to define the diagnostic value of sIgE to peanut components and compare this value with a pediatric population. **Methods:** Validation was performed by discrimination with an area under the receiver operating characteristic curve (AUC) and calibration with the Hosmer-Lemeshow test. The diagnostic value of the peanut components was assessed with the AUC.

Results: Validation of the pediatric model in 94 adults showed poor discrimination (AUC 0.64) but good calibration ($P = 0.48$). sIgE to Ara h 2 was the best diagnostic predictor (AUC 0.76, compared to 0.90 in 100 children). By using a cut-off value with a 100% positive predictive value

(≥ 1.75 kU/l), 28% of patients could be accurately diagnosed. Highest negative predictive value (NPV) was 63%. A higher NPV could not be calculated for any other test. In the children data, a 100% PPV was found at a cut-off value ≥ 5.16 kU/l and was applicable to a similar number of patients (26%). In children also a 100% NPV could be calculated using a cut-off value of < 0.07 kU/l. In this way peanut allergy could be accurately excluded in 24% of the suspected peanut allergic patients.

Conclusion: sIgE to Ara h 2 has the best discriminative ability of all peanut specific components in both adults and children. It can accurately diagnose peanut allergy in 28% (adults) and 26% (children) of patients, respectively, but cannot be used to exclude a peanut allergy in an adult population.

850

Component-resolved diagnostics of peanut allergy in Japanese children

Ito, K¹; Kando, N¹; Nakayama, S²; Tanaka, A²; Borres, MP³

¹Department of Allergy, Aichi Children's Health and Medical Center, Obu, Japan; ²Phadia KK (Thermo Fisher Scientific), Tokyo, Japan; ³Phadia AB (Thermo Fisher Scientific), Uppsala, Sweden

Background: Peanuts are one of the most common foods causing allergic reactions in Japanese children. The storage proteins in peanuts, Ara h 1, 2, 3, contribute to the anaphylactic reactions to peanuts. Oral allergy syndrome (OAS) to peanuts is known to be caused by the Bet v 1 related peanut allergen, Ara h 8. On the other hands, there are many peanut-sensitised (PS) children without allergic reactions to peanuts, mostly due to the cross-reactivity to legume families, such as soybeans. Component-resolved diagnostics (CRD) may help the safe and appropriate diagnosis of peanut allergies (PA).

Method: Serum samples from 35 children with PA (aged 6.7 ± 3.0 years old) and 34

PS children (6.1 ± 3.3 years old) were analyzed sIgE to peanut (F13), soybean (F14), Ara h 1, 2, 3, 8, 9, bromelain (representing CCD) and Bet v 2 (representing profilin). F13/F14 ratio was evaluated to show if sIgE recognises both peanut and soybeans, or predominantly peanuts. The component-specific IgE titer was divided by F13, and the dominant component (DC) was defined as the ratio $\geq 50\%$.

Results: F13/F14 ratio was significantly higher in PA (16.1 ± 17.7) than in PS (2.0 ± 1.8 , $P < 0.001$). Sensitivities of positive sIgE (≥ 0.35 kUA/l) were Ara h 2: 83%, Ara h 1: 49% and Ara h 3: 46%. Two PA patients showed positive sIgE to Ara h 8 (4.53 and 65.1 kUA/l) and one to Ara h 9 (41.57 kUA/l) without sensitisation to Ara h 1, 2, 3 (except one 0.48 kUA/l to Ara h 2). Ara h 2 was found to be the DC in 16 PA patients, but only in 3 PS children. On the other hands, Bet v 2 ($n = 10$) and CCD ($n = 8$) were the DC in PS children.

Conclusion: CRD was expected to contribute not only to the diagnosis of PA, but also to the subtype classification of PA.

851

Comparison of skin prick tests with raw milk, ultra heat treated milk and commercially-prepared cow's milk in atopic children

Harmanci, K¹; İpar, N²; Kanbur, S¹; Kocak, AK¹

¹Pediatric Allergy and Immunology, Eskisehir Osmangazi University, Eskisehir, Turkey; ²Eskisehir Osmangazi University, Eskisehir, Turkey

Background: In recent years, evidence has emerged suggesting that the majority of children with milk allergy can tolerate these food when they are extensively heated (EH). Extensive heating alters the allergenic proteins to which IgE antibodies typically form and allergenicity is attenuated in cases of certain allergens such as milk. We investigated in atopic 500 children whether they have differences in skin

prick test sensitivity. Ultra heat treated cow's milk, commercial extracts of cow's milk and raw milk.

Material and Method: The study included 500 children who consulted our department for any allergic reason (Asthma, Atopic dermatitis, Allergic rhinitis, Urticaria). In this study we performed skin prick test in 500 children with against the three types of cow's milk. The anterior surface of the forearm was used for skin prick testing with commercially-prepared cow's milk, raw milk (Whole milk Unheated and untreated) and ultra heat treated (UHT Whole milk heated at 140 c for 1–2 s,) milk extract testing solutions (Omega Laboratories Limited). Two drops of milk extract, a negative control, and a positive histamine control were applied to the forearm. The drop was pricked using a Hollister-Stier lancetter and the tests were read after 15 min.

Results: Forty-six patients (9.2%) developed a skin prick test positivity to raw milk. 31 patients (6.2%) developed a skin prick test positivity to. Ultra heat treated cow milk. 20 patients (4.0%) developed a skin prick test positivity to commercial extracts of cow's milk. There were 19 cases in which only raw milk skin test was positive. In these cases UHT milk and commercial extracts of cow's milk skin test positivity were not observed.

Conclusion: In our study, the rate of finding positive skin prick test against raw milk was significantly higher than other milks (UHT and commercial preparation). This high rate is also observed in all groups of allergic diseases (Asthma, Atopic dermatitis, Allergic rhinitis, Urticaria). UHT milk and commercial milk protein structure torn down and reduces the possibility of allergic reactions seen. Therefore, searching for cow's milk allergy. We should not only use commercial extracts of cow's milk but also we should use raw milk.

Poster Session 27

Immunology of the gastrointestinal tract and allergic reactions

852

Establishing the use of hypoallergenic formulae with cow's milk protein induced gastrointestinal allergies

Yerlett, NC¹; Meyer, R²; Dziubak, R¹; De Koker, C¹; Dominguez Ortega, G¹; Skrapac, A-K¹; Godwin, H¹; Shah, N¹
¹Nutrition and Dietetics, Great Ormond Street Hospital for Children NHS Trust, London, United Kingdom;
²Gastroenterology, Great Ormond Street Hospital for Children NHS Trust, London, United Kingdom

Background: The mainstay of treatment for food protein-induced gastrointestinal allergy (FPIGA) to cow's milk is the avoidance of cow's milk protein (CMP). In the absence of breast milk, a hypoallergenic formula (HF) is required. No official guidance in the UK exists for appropriate first-line HF in infants with FPIGA. We set out to establish HF use in FPIGA patients.

Methods: Dietetic records of children (0–5 years), seen by the gastroenterology department (2009–2012), who were prescribed HF for suspected FPIGA to CMP, were assessed to establish: HF choice, tolerance, number of feed changes, and number of symptoms. Feed choices were categorised as: amino acid formula (AAF), extensively hydrolysed formula (EHF), and partially hydrolysed formula (PHF).

Results: Sixty-one patients (37 male, median age 16 months (range 2–52 months)) were included. 40/61 (66%) showed total improvement on the chosen HF; The remaining 21 patients showed partial (10/61, 16%) or no improvement (11/61, 18%). Of the 40 children who improved on HF: 14 (35%) were tried on EHF, with success in 8/14. The remaining six improved when switched to AAF. In 14/40 (35%) symptoms resolved when directly prescribed AAF, and 8/40 (20%) improved on an alternative formula. The remaining 10% improved on PHF. Patients who improved on EHF had a median of 4.5 symptoms, and 2 feed changes. However, patients who

failed EHF and subsequently improved with AAF, had a median of three symptoms, with an average of only 1 feed change.

Conclusion: 66% of patients presenting with gastro-intestinal symptoms, had total resolution of these symptoms after the implementation of HF. Resolution of symptoms was achieved in 50% by AAF, 20% by EHF, 20% by other formula, and 10% by PHF. Patients prescribed directly onto AAF had a lower number of feed changes, as compared to those who successfully resolved on EHF, although the latter resolved more symptoms; questioning whether direct prescription of AAF was appropriate in these cases. A tailored care-pathway specific for FPIGA is required. This will guide appropriate, cost-effective, first-line HF prescription choice. Particularly, as the rate of EHF feed failure is 43% in this population.

853

Racial differences in eosinophilic gastrointestinal disease: a systematic review

Ito, J^{1,2}; Fujiwara, T^{1,2}; Nomura, I³

¹Department of Social Medicine, National Research Institute for Child Health and Development, Tokyo, Japan; ²Department of Social Medicine, Mie University Graduate School of Medicine, Tsu, Mie, Japan;

³Department of Allergy and Immunology, National Research Institute for Child Health and Development, Tokyo, Japan

Background: Although empirical evidence showed that the prevalence of eosinophilic esophagitis (EoE) is higher in the Western countries than in Asian countries, whereas eosinophilic gastroenteritis (EGE) is more prevalent in Asian countries than in Western countries, no systematic review investigated the differences. We investigate racial differences of eosinophilic gastrointestinal

disease (EGID) in terms of involved gastrointestinal tract by a systematic review.

Method: We conducted systematic literature using PubMed in September 2012. Studies were excluded non-English, no abstract, or review articles, and those which did not contain information of race except articles from, Japan, Korea, China, and Taiwan. Further, we limit the articles which used single race (at least 95% of sample were same race), either Caucasian or Asian.

Result: Among 687 studies hit in PubMed, 129 studies fulfilled the criteria. In a total of 2619 EGID patients were collected (Caucasian, *n* = 2010; Asian, *n* = 382). About 93% of Caucasian was EoE (*n* = 1873), and the percentage is significantly higher than Asian (*n* = 105, 28%). The same trend was confirmed even after patients were stratified by age, i.e., among children aged less than 21 years old and adults. Percentage of eosinophilic positive esophagus among Caucasian EGID was 87%, which is significantly higher than among Asian (26%). However, percentage of eosinophilic positive stomach, small intestine, and colon among Asian were 27, 48, and 24%, respectively, all of them were significantly higher than among Caucasian (9, 7, and 2%, respectively). Among EoE cases, Caucasian was 3.54 or 3.09 times significantly more likely to have dysphagia or heartburn than Asian, respectively. In contrast, Asian was 5.48 times significantly more likely to vomit than Caucasian. Among EGE cases, Caucasian was 3.07 times significantly more likely to vomit than Asian. There was no statistical difference about abdominal pain and diarrhea between Caucasian and Asian among EGE cases.

Conclusion: We confirmed that in EGID cases, esophagus is more likely to be involved among Caucasian, whereas lower gastrointestinal tract is more likely to be

Table 1: Number of feed changes and number of symptoms

	Median number of feed changes	Median number of symptoms
Improved on EHF	2	4.5
Improved on PHF	2.5	3
Failed EHF, improved with AAF	3	3
Straight to AAF and improved	1	4
Improved on alternative milk (1 modular, 1 soya, 6 coconut/oat milk)	2.5	5

involved among Asian. Furthermore, even in the same disease (EoE or EGE), symptoms are different between these races. The racial differences of specific eosinophilic positive intestinal tract among EGID suggest further genetic and environmental studies for etiology of EGID.

854

Characteristics of infants with food protein-induced enterocolitis syndrome: one center's experiences with 17 cases

Arik Yilmaz, E¹; Cavkaytar, O¹; Buyukiryaki, B¹; Soyer, OU¹; Tuncer, A¹; Sackesen, C¹

¹Department of Pediatric Allergy, Hacettepe University Faculty of Medicine, Ankara, Turkey

Background: Food protein-induced enterocolitis syndrome (FPIES) is a non-IgE-mediated food allergy characterised by profuse, repetitive vomiting, often with watery diarrhea with blood/mucus. Although it is a very rare disease, its prevalence is increasing in the last decade. In this study we aimed to determine the clinical and laboratory features of FPIES in 17 infants who were diagnosed as FPIES.

Method: FPIES was diagnosed in the presence of gastrointestinal symptoms; such as repetitive vomiting, watery diarrhea with mucus or blood, abdominal distention within 24 h after the ingestion of incriminated foods, without any other cause for the symptoms; or a positive open food challenge result with causative food (containing 0.15 gr/kg food protein) or removal of causative food from the diet resulting in the resolution of symptoms in infants with age less than 9 months.

Results: We analyzed 17 patients with FPIES [7 (41.2%) male; 10 (58.8%) female] in last 2 years (December 2010–December 2012). The age of onset of initial symptoms was 2.5 ± 0.5 months (mean \pm SEM). Cow's milk was determined as trigger of FPIES in 16 (94.1%) patients and rice was in 1 (5.9%) patient. Blood in stool was the most common clinical feature ($n = 12$; 70.6%) followed by diarrhea ($n = 11$; 64.7%), vomiting ($n = 9$; 52.9%), mucus in stool ($n = 9$; 52.9%), abdominal distention ($n = 7$; 41.2%), weight loss ($n = 7$; 41.2%) and lethargy ($n = 5$; 29.4%). Three patients had been hospitalised 6 times because of FPIES symptoms. Weight and height percentiles of patients were in normal ranges except one. Only two patients had atopic dermatitis. Total IgE levels were in normal ranges in patients (18 ± 7.6 kU/l) but eosinophil count was slightly high as 666 ± 182.1 . Symptoms resolved with removal of incriminated foods from diet in all patients. Oral food challenge test was per-

formed in 11 (64.7%) patients and 7 of those (63.6%) resulted positive. Only one patient had medical treatment (i.v hydration) at open food challenge.

Conclusion: Our results show that the most common symptoms of FPIES are vomiting and bloody stool. Our observations denoted that cow's milk is the most common trigger and rice may rarely induce the symptoms of FPIES.

855

Retrospective study of patients with eosinophilic esophagitis

Candón Morillo, R¹; Moreno Mata, E²; Burgos Montero, AM²; Ruiz León, BM²; González Sánchez, LA²

¹Allergy Department, Hospital La Mancha Centro, Alcazar de San Juan, Spain; ²Hospital La Mancha Centro, Alcazar de San Juan, Spain

Background: Analyze epidemiological and clinical characteristics and the treatment of the patients, diagnosed of Eosinophilic Esophagitis (EE).

Method: We reviewed the record of 40 patients with EE of our allergy unit.

Results: Patients: 36 adults and four children. 72.5% of patients were male.

Atopic disease: 62.5%

Rhinoconjunctivitis (40%)

Asthma (16%)

Rhinoconjunctivitis and asthma (12%)

Respiratory allergy and food allergy (24%)

Respiratory allergy and dermatitis (5.5%)

-Symptoms:

Adult:

Choking: 30.5%

Dysphagia: 19.4%

Dysphagia + food impaction: 19.4%

Oppression pharyngeal: 16.6%

Epigastric pain: 5.5%

Impactacion: 5.5%

Vomit: 2.7%

Children:

Choking: 50%

Dysphagia: 25%

Stomachache + diarrhea: 25%

-Treatment:

Fluticasone (60%)

No treatment (17.5%)

Fluticasone and proton pump inhibitor (PPI) (12.5%)

Elemental diet and PPI (2.5%)

Conclusions: The media age in our study is 32.3 years old and we observed that males are more commonly affected than females. Atopic disease seems to be a risk factor in EE, mainly rhinoconjunctivitis followed by asthma.

Clinical manifestations of EE differ according to age of patient. Most patients controlled the symptoms with fluticasone.

Elemental diets are effective but difficult to carry out them, so this is the reason that they are little recommended in daily clinical practice.

856

The role of food allergens in eosinophilic esophagitis. Preliminary results

Zande, M¹; Angelakopoulou, A²; Pitsios, K¹; Politi, E³; Kleanthous, K⁴; Panagiotou, I⁵; Roma-Giannikou, E⁵; Syrigou, E¹

¹Allergy, General Hospital 'Sotiria', Athens, Greece;

²Gastroenterology, The Hospital for Sick Children, GOS, London, United Kingdom;

³Cytopathology, Areteio University Hospital, Athens, Greece;

⁴Pediatrics, University Hospital Attikon, Athens, Greece;

⁵Children's Hospital 'Agia Sofia', 1st Paediatric Clinic University of Athens, Athens, Greece

Background: Eosinophilic esophagitis (EoE) is a chronic inflammatory disease of the esophagus immune/antigen mediated. It is clinically characterised by symptoms related to esophageal dysfunction and associated with eosinophil-predominant esophageal inflammation. The role of atopy has been clearly demonstrated both in epidemiological and experimental studies and has important implications for diagnosis and therapy. The aim of this study was to assess the relationship between food allergy and eosinophilic esophagitis in the pediatric population, as well as the effect of dietary modifications in patients' clinical symptoms.

Method: Thirty-six children aged 7 months to 12 years (median age 80 months), with EoE (esophageal symptoms, biopsy with >15 eosinophils/HPF after the patients have been treated with a PPI for at least 8 weeks and other causes have been excluded), were considered as group I. Twenty age and sex matched, apparently healthy, infants and children were studied as control group (group II). Serum specific IgEs to cow milk, egg, wheat, rice, corn, soy, chicken, potato, beef, peanut and pork were measured with the CAP-FEIA. Skin prick tests and atopy patch tests (using fresh foods) were performed for the same allergens.

Results: All children in control group had negative CAP, SPT and APT to all food allergens. In group I 30/36 children (83%) had positive APT. Of these, 12/30 (40%) had also positive SPT and 16/30 (53%) had also positive CAP. Of the 30 positive children 4/30, (13%) had positive APT to one food allergen, 2/30 (7%) had positive APT to two food allergens and 23/30 (77%) to 3 or more food allergens. In the APT-positive children, in group I, withdrawing the suspected food allergens for an 8 week period resulted in the improvement of symptoms.

Conclusion: Food allergens seem to be a significant etiologic factor for eosinophilic esophagitis in infants and young children. Skin tests are able to identify, in most of the cases, the responsible food allergens leading to dietary modifications and symptom remission.

857

The role of allergy in the etiology of functional dyspepsia

Bozkurt, B¹; Nadir, I²; Sözen, M²; Yalcin, KS³; Ikizel, M³; Türkay, C²

¹School of Medicine, Immunology and Allergic Diseases, Fatih University, Ankara, Turkey; ²School of Medicine, Gastroenterology, Fatih University, Ankara, Turkey; ³School of Medicine, Internal Medicine, Fatih University, Ankara, Turkey

Background: Functional dyspepsia (FD) is a condition that causes an upset stomach or pain or discomfort in the upper belly, near the ribs. The exact cause for functional dyspepsia is still unknown. The aim of this study was to evaluate the role of allergy in the etiology of FD.

Method: The study included 100 patient admitted to our Gastroenterology outpatient clinic with the diagnosis of FD between April 2011 and 2012. 79 of these patients were evaluated for the history of allergic diseases. And the skin prick tests (SPT) with common aeroallergens and food allergens were performed.

Results: The mean age of the patients was 42. ± 13.4 years and 62% were male. SPT were positive in 51 of 79 patients. Among these, 21 patients were sensitive to food allergens 43.5% of the patients were active smokers. There were only five patients with mild asthma. Most common allergic diseases were; seasonal allergic rhinitis (51.6%), metal allergy (24.2%), food hypersensitivity (19.4%) and drug hypersensitivity (8.1%). Most common aeroallergen was pollens (49.4%) and cockroach (32.9%). Most common food allergens were peanut (8.9%) and soybean (7.6%).

Conclusion: The allergic diseases were commonly seen in patients with FD. The higher rates of seasonal allergic rhinitis (51.6%) and pollen allergy (49.4%) in FD might show the presence of gastrointestinal involvement in these patients as in oral allergy syndrome. The increased rate of cockroach allergy (32.9%) in FD might be due to contamination of food and food additives with these allergens leading hypersensitivity in gastrointestinal tract. The increased rate of metal allergy (24.2%) in these patients might show hypersensitivity to metal containing foods. The patients diagnosed with FD should be further evaluated for allergic diseases by skin prick tests

for aeroallergens and food allergens and patch tests for metal allergens.

861

Gastrointestinal food allergy assessment by a symptom questionnaire

Dominguez-Ortega, G¹; Meyer, R¹; Dziubak, R¹; De Koker, C¹; Godwin, H¹; Yerlett, N¹; Skrapac, AK¹; Acton, N¹; Shah, N¹

¹Great Ormond Street Hospital, London, United Kingdom

Background: Clinical history and symptoms form the cornerstone for the suspicion of Gastrointestinal food allergy (GIFA), which is confirmed with an elimination diet and challenge. However, symptom improvement following an elimination diet is often ambiguous. The use of a Likert scale GIFA symptom questionnaire (GIFASQ) to aid the decision process has not been studied in GIFA. We therefore set out to determine the impact on symptom improvement of dietary elimination, additional medication or both using the GIFASQ.

Method: This prospective observational study was conducted in a tertiary paediatric gastroenterology department. The GIFASQ was developed from symptoms of 437 children with proven GIFA. Parents completed the questionnaire before commencing the exclusion diet and/or medication and repeated this after 4 weeks. The type of intervention was determined by an expert pediatric gastroenterologist. The GIFASQ measured nine symptoms individually from 0 (no symptoms) to 5 (most severe) and globally from 0 to 45.

Results: Data from 77 participants was analysed, median age 29.9 months, male 51 (66.3%). Fifty three patients (69%) required only a dietary elimination, 14 (18%) additional medications and 10 (13%) diet and medications. The majority were on different combinations of milk, soy, egg and wheat free diets and four

required further elimination. The most frequently used medications were Ketotifen (15, 62%) and Nalcrom (13, 54%). Forty five patients (58%) achieved ≥ 50% improvement and 5 (6.5%) total improvement in global score after intervention. From the 14 prescribed additional medications, 12 showed further improvement.

Conclusion: The GIFASQ aids in assessing symptom improvement objectively in suspected GIFA. It is a quick and easy tool that can be used in clinical practice in a condition that is difficult to diagnose. It also helps identify children who only partially respond to an elimination diet and who could benefit from the addition of adjuvant medications.

862

Clinical characteristics of gastroallergic anisakiasis: a case-control study in Jeju

Lee, JJ¹; Lee, JH¹; Kim, M¹; Chung, Y-B²

¹Internal Medicine, Jeju National University, Jeju, Korea; ²Parasitology, Jeju National University, Jeju, Korea

Background: Acute gastric anisakiasis is an incidental gastrointestinal infestation of third-stage *Anisakis* spp. larvae (anisakis), which is suggested as one of the causative substances for food hypersensitivity. In the recent literatures, it is called as gastroallergic anisakiasis. However, it is still in doubt whether it is one of the independent disease entities, which can be discriminated from acute gastric anisakiasis. We investigated clinical and immunological features, diagnostic implications of anisakis-specific IgE testings and IgE binding components.

Method: In a geographic region where eating of raw marine seafood is common and habitual in public, a prospective case-control study was designed. Case subjects clinically diagnosed of gastroallergic anisakiasis were selected; clinical presentations of hives, swollen face, abdominal pain, shortness of breath, or diarrhea

Table 1: Individual symptom improvement for all patients

Symptom	Deteriorate	No Change	a) Partial improvement	b) Total Improvement	Improvement a) + b) n (%)
Abdominal Pain	3	16	38	16	54 (74)
Flatus	2	17	28	19	47 (71)
Bloating/ Distension	5	12	18	19	37 (68)
Diarrhoea	6	6	24	13	37 (75)
Costipation	4	7	13	23	36 (77)
Food aversion	5	6	19	15	34 (75)
Vomiting	1	6	7	28	35 (83)
Back Arching	1	4	17	20	37 (88)
Rectal bleeding	2	1	4	16	20 (87)

within hours of raw seafood ingestion, and when the causative substance is not identified. Control subjects who never experienced those symptoms among raw seafood lovers were screened by a questionnaire. Clinical and immunologic features were compared in atopic status, sensitisation rates to anisakis and serum titer of anisakis-specific IgE and IgG.

Results: Seventeen cases and 135 controls were enrolled. The cases experienced gastrointestinal symptoms after raw seafood ingestion, followed by mucocutaneous, respiratory, and multi-systemic symptoms. Their raw seafood dishes were reported diverse. Cases were more frequently sensitised to both anisakis excretory secretory product (ESP) and crude extracts than controls. (76.4% vs. 28.1%, 88.2% vs. 36.3%, respectively) Cases showed significantly higher titer of serum anisakis-specific IgE. Detection of sensitisation by skin prick tests or serum specific IgE with titer might be useful diagnostic testing. Some IgE binding components identified by western blot might have diagnostic implications.

Conclusion: Gastroallergic anisakiasis is a food hypersensitivity to live *Anisakis* spp. larvae. Among the patients suspicious of gastroallergic anisakiasis, the diagnosis might be strongly supported by detection of anisakis-specific IgE. Once gastroallergic anisakiasis is diagnosed, a medical treatment for food hypersensitivity is cautiously suggested as a treatment option other than upper endoscopic removal.

864

Gluten-related disorders: basophil activation test may help in differential diagnosis?

Boni, E¹; Scacchetti, AT²; Pepe, P³

¹Dermatology, University of Parma, Azienda Ospedaliero-Universitaria Policlinico di Modena, Modena, Italy; ²Department of Clinical Pathology, NOCSAE, Modena, Italy; ³Dermatology, Azienda Ospedaliero-Universitaria Policlinico di Modena, Modena, Italy

Background: The main forms of gluten-related disorders are wheat allergy (WA), autoimmune disease (celiac disease, dermatitis herpetiformis and gluten ataxia) and possibly immune-mediated disease (gluten sensitivity). IgE antibodies play an essential role in the allergic disease. For celiac disease (CD) the available tests are anti-tissue transglutaminase (tTG) IgA, anti-endomysial antibodies (EMA) and deamidated gliadin peptides (DGP) antibodies especially IgG class. For other immune-mediated disorders there is currently no available test. There is evidence that the basophil activation test (BAT) reveals IgE and non-IgE

mediated reactions. The BAT based on CD63 detection on whole blood sample showed significant higher sensitivity and specificity and diagnostic accuracy compared to the assay based on separated leukocytes.

Method: We present a case of a woman with wheat-related disorder manifesting clinical signs, intestinal symptoms (chronic diarrhea, weight loss) and extraintestinal symptoms (chronic urticaria-angioedema, fatigue). Skin Prick test (SPT) (Lofarma), *in vitro* specific IgE (Thermo Fisher Scientific), DGP IgG and tTG IgA antibodies (Thermo Fisher Scientific), EMA (Eurospital), HLA (Euroimmune) were investigated. The BAT (Beckman Coulter) with wheat extract was also performed. The patient underwent endoscopy of the digestive tract.

Results: SPT and serum s-IgE assays excluded wheat allergy: SPT was negative for wheat; S-IgE: f4 ≤ 0.10 kUA/l, f79 ≤ 0.10 kUA/l, g2 ≤ 0.10 kUA/l, g8 = 0.54 kUA/l. DGP IgG and tTG IgA, AGA IgA/IgG, EMA were in range of normality. HLA-DQ2/DQ8 was negative. Normal digestive endoscopy was documented. The BAT (52.9%; c.o. positive ≥ 15%) demonstrated a stimulation of the basophils exposed to wheat extract.

Conclusions: The patient was recommended to undergo a strict gluten-free diet. Shortly after diet, the patient's clinical condition improved. All the data excluded an allergic or autoimmune disease. The fact that the clinical signs and symptoms completely resolved after the diet, demonstrates the implication of gluten exposure in the pathogenesis. We diagnosed gluten sensitivity (GS) on the basis of the algorithm for the differential diagnosis of gluten-related disorders, including CD, GS and WA proposed by Sapone *et al.* In this case, we believe that BAT confirms a hypersensitivity reaction to wheat not IgE-mediated not CD. Further studies will have to demonstrate the mechanism through which wheat plays a pathogenic role.

865

A case of eosinophilic colitis causing partial obstruction of colon

Lee, SM¹; Park, S²; Kang, SM¹; Kim, YJ¹; Kyung, SY¹; Park, J-W¹; Jung, SW¹; Lee, SP¹

¹Department of Medicine, Gachon University Gil Medical Center, Incheon, Korea; ²Ewha Womans University, Mokdong Hospital, Seoul, Korea

Background: There are many documents regarding eosinophilic colitis, but eosinophilic colitis causing total or partial obstruction of colon has been rarely reported. We report a case of eosinophilic

colitis causing partial obstruction of colon.

Case report: A man aged 72 years had difficulty in defecation for a month. He received intermittent enema, but symptom was aggravated. He underwent colonoscopy, abdominal computed tomography (CT), and angiography of inferior mesenteric artery (IMA). Colonoscopy showed an ulcerative lesion approximately 40 centimeter above the anal verge. Abdominal CT showed thickened wall of proximal sigmoid colon and rectum with diffuse fecal retention, but without definite transitional zone. Angiography of IMA did not yield significant stenosis or occlusion. IMA-preserving sigmoidectomy was performed, and pathologic study revealed chronic active inflammation with many eosinophil accompanied by ulcer and seven reactive pericolic lymph node. Peripheral blood eosinophil was normal range (96 /μL) before operation, but mildly increased to 581 /μL six weeks later. Total serum IgE, and eosinophilic cationic protein (ECP) were also elevated (904 U/ml and 17.8 μg/l, respectively)

Conclusion: We might consider the possibility of eosinophilic colitis when the thickened wall of colon causes subacute partial obstruction of colon accompanied by the elevation of peripheral blood eosinophil, total serum IgE, or ECP.

866

Rice sensitisation in patient with eosinophilic esophagitis

Flores Martín, IM¹; Peña Arellano, MI¹; Miras Bruno, JA¹; Expósito Barroso, F²

¹Allergy, Hospital Vega Baja, Orihuela, Spain; ²Hospital Vega Baja, Orihuela, Spain

Background: Eosinophilic esophagitis represents a chronic, immune/antigen-mediated disease characterised clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation.

Method: A 51 years old man was referred for allergy review after being diagnosticated Eosinophilic esophagitis by gastroenterology. The patient had suffered in the last year several episodes of food impaction and a prolonged history of dysphagia after eating.

Upper gastrointestinal endoscopy was undertaken with two esophageal biopsies, finding

Basal cell hyperplasia, edema of squamous epithelium and intraepithelial eosinophils.

The patient fulfilled the diagnostic criteria for EOSINOPHILIC ESOPHAGITIS

The patient began treatment with swallowed fluticasone without resolution of symptoms.

In allergy anamnesis the patient referred dysphagia symptoms with bread, chicken and rice. Skin prick test with a large food battery was performed with rice positive result.

Results: The patient was treated with rice free diet and continued with swallowed fluticasone. One month after treatment the symptoms disappeared. The patient reintroduced rice diet resubmitting dysphagia. Actually the patient is asymptomatic with rice free diet and swallowed corticosteroid.

Conclusion: Eosinophilic esophagitis prevalence is increasing. Therapeutic options include:

- * Chronic dietary elimination
- * Topical corticosteroids
- * Esophageal dilatation

It is important to identify food sensitisation in order to improve the symptoms without needed aggressive therapies. The reversibility of esophageal remodelling and subepithelial fibrosis with standard treatments is still unknown, but it is possible that an earlier diagnosis in the current patient might have resulted in earlier treatment and preventing stricture formation.

867

Gastrointestinal manifestations in children with respiratory allergy

Bakiri, AH¹; Mingomataj, EC²

¹Allergology Department, Hygeia Hospital Tirana, Tirana, Albania; ²Allergology Department, Mother Theresa School of Medicine, Tirana, Albania

Background: Although there are few investigations reporting gastrointestinal (GI) manifestations in asthmatic children like diarrhea, vomiting, abdominal pain, etc., little interest is shown on their relationship. In some studies are demonstrated duodenal histological changes similar to those of bronchial mucosa.

Case reports: Hereby we will present two cases. The first one presents a boy of 11 years old who came in our hospital with episodic abdominal pain. He referred to have had allergic rhinitis and mild symptoms of atopic dermatitis, while his aunt suffers from allergic rhinitis. He has been treated for the abdominal pain with analgesics and myorelaxants, antihistamines and nasal corticosteroids also. In the second case we will report the history of a 12 years old girl who suffered from asthma since from her childhood. The girl arrived to hospital without asthma symptoms but abdominal pain. In the second episode the girl complained of intermittent pain that begun a day before. Lung auscultation

revealed sibilance. The GI manifestations were present right after her classroom is being cleaned. The patient begun regular treatment with inhalant corticosteroids, β_2 agonists and subcutaneous immunotherapy (without abdominal pain related to the injection) 2 years ago. The patient suffered from atopic eczema and referred of mild regurgitation episodes also.

Methods and Results: Physical and laboratory examinations were within normal range. Skin prick test revealed sensitisation to house dust mite for both patients. The girl atopy patch test resulted positive for mites.

Discussion: There might be several reasons for the relationship between asthma GI symptoms like: asthma itself induces GI symptoms, drugs like glucocorticoids and β_2 agonists decrease the esophageal sphincter pressure or the inhalation of the allergen may induce abdominal pain.

Conclusion: GI symptoms appear to be common in atopic children with asthma but further studies are needed to shed light on this relationship.

869

Eosinophilic esophagitis in a Mediterranean area

Mérida-Fernández, C¹; Ramírez-Hernández, M¹; Pajaron-Fernández, MJ¹; Carreño-Rojas, A¹; Huertas-Amorós, AJ¹

¹Allergy, Complejo Hospitalario Universitario de Cartagena, Cartagena (Murcia), Spain

Background: Eosinophilic esophagitis is defined as a chronic, immune/antigen-mediated esophageal disease characterised clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation. It usually affects children and adults in their 20s or 30s and it is more common in males. The majority of patients has the concurrent allergic disease, especially food or aeroallergen sensitisation.

Method: We describe 14 patients (9 (64%) males and 5 (36%) females) who were diagnosed with eosinophilic esophagitis.

The mean age was 33.2 years.

Skin prick test with foods were done in all patients.

Skin prick test with aeroallergens were done in 11 patients (three patients did not complete the study).

Results: Dysphagia (42.8%) and food impaction (42.8%) were the most common symptoms referred by the patients.

11 (78.5%) patients were sensitised to any food. Allergies to dry fruits (63.6%), paprika (45.5%) and peach (36.4%) were the most common.

Four out of 11 patients sensitised to any food, referred symptoms in relation with

the ingestion of the food positive in skin prick test.

6 (42.8%) patients had associated rhinitis and/or asthma.

Skin prick tests positives to any aeroallergen were found in 8 (57%) patients. Pollen (81.8%) was the most common aeroallergen followed by dust mites (27.3%).

Conclusion: Eosinophilic esophagitis is a relatively new entity with a significant increased recognition over the last decade.

In our population:

The majority of patients were sensitised to dry fruits, paprika and peach.

Pollen is the aeroallergen more common.

Rhinitis and/or asthma were associated in 42.8%.

870

Histamine modulates toll-like receptor responses in peripheral blood mononuclear cells from inflammatory bowel disease patients

Smolinska, S¹; Konieczna, P²; Jutel, M¹; O'Mahony, L²

¹Department of Clinical Immunology, Wrocław Medical University, Wrocław, Poland; ²Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland

Background: Histamine plays an important role in chronic inflammatory responses due to its immune-modulatory effects. Histamine exerts its functions through its four different receptors HR1-HR4. Toll-like receptors (TLRs) are involved in innate immune response due to their ability to recognise PAMPs (pathogen associated molecular patterns) and are essential for protection against infection.

Method: Fourteen patients diagnosed with IBD, seven with Crohn's disease (CD) and seven with ulcerative colitis (UC), and six healthy volunteers were investigated. Peripheral blood mononuclear cells (PBMCs) were isolated from all subjects and gene expression for TLRs and histamine receptors (H1R, H2R, H4R) were quantified by RT-PCR. In addition, PBMCs were stimulated with various TLR ligands in the presence or absence of histamine. Cytokine levels (IL-12, TNF- α , IP-10) were determined in culture supernatants after 24 h stimulation.

Results: PBMCs from UC and CD patients showed significantly reduced gene expression of TLR-1, TLR-2, TLR-4, TLR-6 and TLR-9 compared to healthy volunteers. Similarly, the expression of histamine receptors H1R, H2R and H4R were significantly lower for the IBD patients (particularly in the CD group). PBMCs from IBD patients secreted significantly more pro-inflammatory cytokines,

compared to healthy volunteers, after stimulation with each of the TLR ligands. Within the IBD group, cytokine secretion was higher in patients with CD compared to patients with UC. In the presence of histamine, TLR-stimulated pro-inflammatory cytokine secretion from PBMCs was significantly reduced for both IBD patients and healthy volunteers. Histamine suppression of cytokine secretion by PBMCs from IBD

patients tended to be less effective as that seen for healthy volunteers. Mean suppression of the LPS-stimulated IL-12 secretion was 90% for healthy volunteers, 65% for CD patients and 74% for UC patients, $P = 0.08$).

Conclusion: PBMCs from IBD patients display significant alterations in the expression of both TLRs and histamine receptors genes, which are associated with a stronger

pro-inflammatory cytokine response to TLR-ligands. Histamine-stimulation effectively dampens pro-inflammatory TLR-associated responses in IBD patients. Further examination of this anti-inflammatory effect may contribute to the development of new therapeutic strategies in chronic inflammatory bowel disorders.